

Strategies to Improve Protein Quality and Reduce Antinutritional Factors in Mung Bean

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ABSTRACT

Legume seeds contain 20-25% protein, which is 2-3 times higher than the content in cereals, and have therefore been considered as leading candidates for protein supply to malnourished areas of the world. Mung beans are a good source of energy, proteins, vitamins, minerals and dietary fiber. They are relatively inexpensive compared to meat foods and as they have high carbohydrate content. Mung beans can be useful as high energy foods for peasants and nomadic farmers. The high lysine content of mung beans makes them an excellent enhancer of protein quality when combined with cereal grain proteins. However, like other pulses, utilization of mung bean proteins is below the potential partly due to the deficiency of some of the essential amino acids in their proteins and also due to the presence of some antinutritional factors associated with their protein. Protease inhibitors, lectins, gossypol, flatulence-causing factors and others are among the antinutritional factors associated with the legume meal proteins. We report here many investigations carried out in order to reduce or even remove the content of antinutritional factors and enhance the nutritional quality of mung beans. Finally, we discuss the possibilities of mung bean flour to improve and upgrade the nutritional quality of the diets and the health of low-income people in developing countries.

Keywords: antioxidative activity, legumes, nutrient composition, Vigna radiata

Abbreviations: AL, available lysine; ANF, antinutritional factor; BHT, butylated hydroxytoluene; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GC, gas chromatography; GI, glycemic index; GIT, gastrointestinal tract; HHP, high hydrostatic pressure process; IVPD, *in vitro* protein digestibility; MS, mass spectrometry; PA, phytate; PEM, protein-energy malnutrition; PER, protein efficiency ratio; RDA, recommended dietary allowance; RFO, raffinose family oligosaccharides; RS, resistance starch; SAA, sulfur amino acid; SSB, solid state bioconversion; SSF, solid state fermentation; TI, trypsin inhibitor; TN, tannin

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INTRODUCTION

In tropical and third world countries an acute shortage of protein-rich foods due to population explosion and cost effectiveness of animal-based protein has resulted in an overwhelming interest in underutilized proteinaceous wild leguminous plants. Underutilized wild legumes are of immense value due to their low cost, high nutritional value and presence of health-promoting bioactive compounds (Bhat *et al.* 2007a). Grain legumes are considered to be good for health due to their mutual compatibility with cereals and for their properties in disease prevention, including cardiovascular diseases, type 2 diabetes, obesity and, possibly, colon cancer (Guillon and Champ 2002). The nutritional potential of the seeds from this group of plants is based on their high level of protein. Along with macronutrients, leguminous seeds contain appreciable amounts of some vitamins and minerals as well as dietary fibre (Saikia *et al.* 1999). The most common legumes for human consumption are bean, lentil, pea, chickpea and faba bean (Guillon and Champ 2002). Among the legumes, mung beans have been found to be promising sources of proteins and essential amino acids.

Mung bean (*Vigna radiata* var. *aureus*), an Asian plant in the pea family, is widely cultivated for its edible seeds and pods. It is the chief source of bean sprouts. Mung beans, a small dried bean with yellow flesh and a skin that is normally green but sometimes yellow or black. Mung beans are an important constituent of human diets in central, southern and eastern Asia and have been cultivated in this region for centuries (Wikipedia 2008). More recently, mung beans have become widely used in the tropics and sub-tropics of Africa, the West Indies, North America and Australasia (Liu and Shen 2007).

Mung bean is similar in composition to other members of the legume family, with 24% protein, 1% fat, 63% carbohydrate and 16% dietary fiber (US Department of Agriculture 2001). Mung bean is an excellent source of vitamins, minerals and protein with its essential amino acid profile comparable to that of soybean and kidney bean (Mubarak 2005). Recent research indicates that mung bean consumption produces a small increase in blood glycemic index in humans making it an attractive option for diabetic patients. It is reported to modify glucose and lipid metabolism favorably in rats (Bornet et al. 1989; Lerer-Metzger et al. 1996). Therefore identification and utilization of anti-diabetic plants that contain phenolic substances/ α -amylase inhibitors which interact with digestive enzymes thereby modulating their activity is beneficial (Alarcon-Aguilar et al. 2005). Hence, food sources with a hypoglycemic effect and a high antioxidant activity such as mung bean are beneficial for diabetics (Randhir and Shetty 2007). It is also well documented that certain proteins in mung bean exert both antifungal and antibacterial activity (Wang et al. 2004). The antimicrobial potential of mung bean sprout extracts to inhibit the growth of the bacteria Helicobacter pylori that causes gastro duodenal disease was confirmed (Mitchell and Megraud 2002; Randhir et al. 2004; Randhir and Shetty 2007).

The genus Vigna consists of several related species which have been cultivated in central, southern and eastern Asia for a very long time. The two most important species in the genus Vigna are cowpea (Vigna unguiculata), an important grain legume of the African lowland tropics and mung bean (*V. radiata*), which is grown extensively in India and Southeast Asia. The taxonomic history of the Asiatic Vigna species has been confused at both generic and specific levels. The group was formerly placed in the genus *Phaseolus*, but it is now generally accepted that it belongs in the genus Vigna. The naming of these two species has been very unclear in the literature to date. Unless the common names are accompanied by the appropriate scientific names, it is very difficult to differentiate between the two Vigna species and consequently important scientific data cannot be properly attributed to the correct variety. Debate continues as to whether var. *aureus* and var. *mungo*, which share many morphological similarities, are indeed separate species. Verdcourt (1970) considers that these two groups are in fact variants within one species, but he has recommended the retention of the separate designations. There is no doubt that the two species \hat{V} . radiata var. aureus and V. radiata var. mungo are close relatives. Urd bean (black gram, V. mungo), is a more recent crop, but it is an important pulse crop in India, Burma, Bangladesh, Pakistan and Thailand and of minor importance in Sri Lanka. It is grown in a limited scale in other parts of Southeast Asia, Australia and Fiji (Savage 1990).

Although legumes constitute one of the most abundant and least expensive sources of protein in human/animal diets, their utilisation is limited largely due to the presence of antinutritional/antiphysiological compounds (Liener 1994), including protease inhibitors (trypsin), α -amylase inhibitors, lectins, polyphenolic compounds, tannins (TN), phytic acid (PA), flatulence factors, and allergens (Liener and Kakade 1980; Liener 1989). These factors negatively affect the nutritive value of beans through direct and indirect reactions: they inhibit protein and carbohydrate digestibility; induce pathological changes in intestine and liver tissue thus affecting metabolism; inhibit a number of enzymes and bind nutrients making them unavailable (Bressani 1989, 1993). In order to make food grain legumes edible, consumers have developed a relatively large number of processing methods. Researches have studied these traditional recipes, as well as some more modern technologies, and the results obtained have helped not only to make the beans more acceptable to consumers but also to remove or significantly reduce any antinutritional elements in the food grain legumes. This review was initiated to give detailed information of the nutritional and cooking quality, antinutrients and recommended processing methods to remove/reduce antinutritional and flatulence-causing factors and increase protein digestibility with special concern of mung beans.

NUTRIENT COMPOSITION OF MUNG BEANS: OVERVIEW OF THEIR NUTRITIONAL PROFILES AND HEALTH BENEFITS

The nutritional value of legumes as sources of protein and carbohydrates in the diet is undeniable. Beans are a rich and inexpensive source of proteins (20-25%) and carbohydrates (50-60%) for a large part of the world's population, mainly in developing countries (Rehman et al. 2001), being considered as poor man's meat. The energy content of most legumes has been found to be between 300 and 540 Kcal/100 g (310 Kcal/100 g of mung bean). Energy is required for all metabolic processes. The energy of pulses comes from the nutrient supply of protein, fat and carbohydrate (Ofuya and Akhidue 2005). Mung beans are a good source of vitamins A, B, C and E, calcium, iron, magnesium, potassium, and amino acids. Mung beans contain 20% protein and are a good source of foliate and dietary fiber. The overall composition of mung beans is similar to that of other grain legumes, with 24% protein, 1% fat, 63% carbohydrate and 16% dietary fiber (US Department of Agriculture 2001). They lack sulfur-containing amino acids and uncooked beans contain trypsin inhibitors (TIs) (Khader and Rao 1996).

Protein

Pulses have a high protein content, the value being about twice that in cereal and several times that in root tuber (FAO/WHO 1973), so they can help to improve the protein intake of meals in which cereals and root tubers in combination with pulses are eaten. Pulse, when eaten with cereals, can also help to increase the protein quality of a meal. In man, protein helps in the repair of body tissue, synthesis of enzymes and hormones and also in the supply of energy. In children, the consumption of pulses should be encouraged, particularly where animal protein is scarce and expensive, as this would help to furnish the child with the necessary amino acids required for growth. The protein content of beans is generally between 20% and 30% of energy. A serving of beans (\approx 90 g or 1/2 cup cooked beans) provides \approx 7– 8 g protein or $\approx 15\%$ of the recommended dietary allowance (RDA) for protein for a 70-kg adult (National Research Council 1989; Messina 1999). Furthermore, the relatively low sulfur amino acid (SAA) content of beans may actually provide an advantage in terms of calcium retention. The reported hyper calciuric effect (excretion of calcium in the urine) of protein is likely to be at least partially due to the metabolism of SAAs (Messina 1999). The skeletal system serves as one of the main buffering systems in the body; as a result, the hydrogen ions produced from the metabolism of SAAs cause demineralization of bone and excretion of calcium in the urine (Chan 1974; Remer and Manz 1994). Human studies showed that the consumption of bean protein is associated with a markedly lower urinary calcium excretion compared with the consumption of similar amounts of whey protein (Anderson *et al.* 1987) or a mixture of animal proteins (Breslau *et al.* 1988). Thus, bean protein may improve calcium retention relative to animal and grain proteins (Messina 1999).

The small seeded tropical Asian beans of the so-called mung group (*Vigna* species), mung bean (green or golden gram, *V. radiata*), and urd bean (black gram, *V. mungo*), and their wild progenitor *V. sublobata* (wild bean), were assessed by Khalil and Khan (1995) for their nutritional composition and protein quality. The protein content of these legumes varied from 20.7 to 23.8%. They were rich in lysine and the aromatic amino acids and limited in S-containing amino acids. Tryptophan was the second limiting amino acid. The protein score ranged from 40 to 64% of the reference protein recommended for child nutrition. In the adult human diet beans can fulfil the requirements of all essential amino acids and thus can serve as supplemental sources of protein in cereal-based diets.

Fat

The fat content of legumes varies in different species. Most species contain about 1% fat, generally containing $\approx 5\%$ of energy as fat. The primary exceptions are chickpeas and soybeans, which contain $\approx 15\%$ and 47% fat, respectively. The fat content, besides contributing to the energy needs, provides the needed essential fatty acids for man. The predominant fatty acid in beans is linoleic acid, although beans also contain the n-3 fatty acid, α -linolenic acid (US Department of Agriculture, Nutrient Data 1988). n-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are being studied for their health benefits (Caygill et al. 1996; Nair et al. 1997; Stone 1997; Simopoulos 1999). Adequate DHA status is particularly important for infants (Oski 1997). α-Linolenic acid can be converted into EPA and EPA can be converted into DHA, although the rate of conversion of α -linolenic acid into EPA is relatively inefficient (5-10%) (Indu and Ghafoorunissa 1992; Emken et al. 1994), and is inhibited by linoleic acid (Emken et al. 1994). The dietary ratio of n-6 to n-3 fatty acids among vegetarians (Messina and Messina 1996) is at the high end of the rather conservative recommendations by the WHO (WHO/FAO 1995).

Micronutrients

Most measures of health in the developing world have shown gradual improvement over the last 50 years. Micronutrient deficiencies (especially iron) have become more common however, even in developed countries. Cereals normally make up the bulk of diets composed of basic grains and supply the greater energy component. Legumes on the other hand contribute more of the other components of diet. Legumes are much more superior to cereals as sources of micronutrients (Welch *et al.* 2000; Broughton *et al.* 2003) first because legumes have a higher initial content of minerals, and second since many cereals are polished before eating.

Minerals

As a significant proportion of the minerals are found in the seed coat (or bran) they are discarded during processing. Most legumes, including common beans, are consumed whole. As a result their mineral content is conserved. Beans provide 10–20% of the adult requirement for a number of nutrients (Broughton *et al.* 2003). Legumes are rich in folate, iron, zinc, and calcium contents. Beans are an excellent source of folate, which in addition to being an essential nutrient is thought to reduce the risk of neural tube defects (Daly *et al.* 1995). One serving of beans provides more than half of the current RDA for folate (National Research Council 1989) and ≈ 2 mg iron. However, iron bioavailability

from legumes is poor and thus their value as a source of iron is diminished (Lynch *et al.* 1984). In contrast to iron bioavailability, zinc bioavailability from legumes is relatively good at $\approx 25\%$ (Sandström *et al.* 1989). Also, many beans are good sources of calcium, providing on average ≈ 50 mg Ca/serving, although there is quite a bit of variation among the legumes. Calcium bioavailability from beans in general is $\approx 20\%$, which is lower than that from milk and green leafy vegetables but is still reasonably good (Weaver *et al.* 1993; Messina 1999).

Vitamins

The vitamins present in appreciably quantities in pulses are thiamin, riboflavin, pyridoxine and folic acid; vitamin E and K are also found in pulses. The B-vitamins act as coenzymes in biological processes. Vitamin E is known to play a role as an antioxidant inhibiting the oxidation of vitamin A in the gastrointestinal tract (GIT) and of polyunsaturated in the tissues. It is also believed to maintain the stability of cell membranes (Davies and Stewart 1987). Vitamin K functions primarily in the liver where it is necessary for the formation of blood clothing factors (Ofuya and Akhidue 2005).

Fiber and the glycemic index (GI)

Legumes are generally good sources of slow-release carbohydrates (Tharanathan and Mahadevamma 2003). Legumes, including mung beans, are beneficial for health, with a low glycemic index (GI; Foster-Powell and Brand-Miller 1995). The high-GI diet stimulates fatty acid synthase activity and lipogenesis, and might have undesirable long-term metabolic effects (Kabir et al. 1998, 2000). Of them, starch and non-starch polysaccharides (dietary fiber) are the major constituents, with a small but significant amount of oligosaccharides (Bravo et al. 1998; Guillon and Champ 2002). Legume starch and fibre both have useful functional properties and can be used readily in food products. Different procedures have been developed for isolating these fractions. Dry and wet separation processes have been used to fractionate grain legumes for experimental purposes and industrial applications (Czukor et al. 2001; Guillon and Champ 2002). Legume seed starch is a source of resistance starch (RS), which seems to be interesting for the production of a large amount of butyrate on its fermentation by colonic bacteria which may help in colon cancer prevention. Raw and processed legumes contain significant amounts of RS in comparison with other products such as cereals, tubers and unripe fruits (Jenkins et al. 1982; Tovar et al. 1992a; Tovar and Melito 1996; Velasco et al. 1997; Bravo et al. 1998, 1999). For this reason, the starch digestion rate and, therefore, the release of glucose into the blood stream are slower after the ingestion of legumes, resulting in a reduced glycemic and insulinemic postprandial responses in comparison with cereal grains or potatoes (Jenkins et al. 1982; Tovar et al. 1992b). Mung bean starch contains about 11% of RS, and to a long absorption period of mung bean starch which was probably not finished 4.5 h after the meal (Guillon and Champ 2002)

In addition to starch, fibre can be extracted from a variety of grain legumes and used as ingredients for food. The physico-chemical characteristics of fibre fractions very much depend on their origin, outer fibres being very cellulosic whereas inner fibres contain a majority of pectic substances. Inner fibres are often used as texturing agents whereas outer fibres find their main uses in bakery and extruded products, where they can be introduced to increase the fibre content of the food. Fibre provides a broad range of positive effects, both physiological and metabolic, at least in subjects suffering from disorders. These effects are related to the source of fibre (from cotyledon or hull), are dose related and depend on the form in which it is ingested. The high dietary fibre content of pulses (15.2 g/100 g of whole mature seeds of mung beans), are postulated to have some important physiological effects, such as reducing the transit time in the mammalian gut (Kamath and Belvady 1980; Sathe *et al.* 1984). This would help to relieve gastro-intestinal conditions such as constipation and diverticular disease. It is also capable of lowering the blood cholesterol level due to its ability to bind with cholesterol in the human gut (Burkitt and Trowell 1985). This feature is being suspected as being capable of reducing colonic cancer in man (Davies and Stewart 1987; Hangen and Bennink 2002).

 α -Galactosides can be isolated during wet processes from the soluble extract. These oligosaccharides are quite characteristic of grain legumes and are present in all species, with large variabilities among different varieties. Overall α galactoside content is within the range of 2-10 g/100 g drymatter (Oboh et al. 1998). a-Galactosides are derived from sucrose and contain 1–3 units of galactose linked by α -1,6 linkages. They are highly soluble in aqueous media and very rapidly fermented by colonic microflora. α-Galactosides are oligosaccharides which are not digested in the upper part of the gastrointestinal tract, due to the absence of α galactosidase among human endogenous enzymes. α-Galactosidase is required to cleave the α -(1-6) galactose linkage present in galactoside-containing oligosaccharides, such as raffinose and stachyose. These oligosaccharides pass into the large intestine and are therefore available for bacterial fermentation in the colon. Due to their high fermentability, α -galactosides induce the production of gases (mainly carbon dioxide, hydrogen, and sometimes methane) responsible for the digestive discomfort and also claimed to be solely responsible for flatulence. Flatus production may be a more acute problem in individuals with colonic pathologies such as irritable bowel syndrome. In fact, for these reasons researchers in Japan have actually suggested that soybean oligosaccharides be used as a substitute for common table sugar (Hata et al. 1991).

In general, beans are an excellent source of dietary fiber; 1 serving provides 2-4 g of a mix of soluble and insoluble fiber (Marlett 1992; Messina 1999). High fiber, high-bean diets were shown to lower serum cholesterol in hypercholesterolemic individuals (Anderson et al. 1984). In addition, beans have very low GIs (Jenkins et al. 1980). This has been attributed to many factors including their fiber (Thorne et al. 1983), tannin (Thompson et al. 1984), and PA contents (Yoon et al. 1983). Although neither the American Diabetes Association nor the American Dietetic Association endorse the GI as a tool for constructing diets for individuals with diabetes (Nutrition recommendations 1994), research published during the past decade makes a persuasive argument that the GI of foods is one factor affecting the overall quality of the diet (Wolever 1997). Thus, beans may be a particularly important food for individuals with diabetes and those with an elevated risk of developing diabetes (Messina 1999).

Antioxidative activity

Naturally occurring antioxidants, such as vitamin C and vitamin E, as well as phenolic compounds, possess the ability to reduce oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases, aging, and brain dysfunction (Yagi 1993; Pietta et al. 1998). Much work has been done to find safe and potent natural antioxidants from various plant sources. Many natural antioxidants are found in the seeds, beans, and the nuts of plants (Namiki 1990). Plant-based antioxidants such as tocopherols, vitamin C, cartenoids, and phenolic compounds are known to protect the plants against oxidative stresses (Packer et al. 1999). Among plants, various beans have been investigated to determine whether they possess antioxidative activity (White and Xing 1997). Several studies reported that bean constituents possessed antioxidative activities in various model systems (Tsuda et al. 1993; Duh et al. 1997). Beans also contain many volatile components (Buttery et al. 1975) which may possess some antioxidative

activity. Aroma extracts isolated from mung beans have been shown to have potent antioxidant activity comparable to that of the known natural antioxidant vitamin E (Lee *et al.*) 2000). Aroma compounds that possess antioxidant activity contained in mung bean was extracted and identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The major aroma constituents of mung beans were alcohols (hexanol, benzyl alcohol and pentanol), lactones (γ -butyrolactone), aldehydes (2-methyl-2-propenal) (Lee and Shibamoto 2000). These aroma compounds exhibited varying amounts of antioxidative activity comparable to those of known antioxidants, butylated hydroxyltoluene (BHT) and R-tocopherol but were less potent. However, these aroma chemicals are present in tremendous numbers. Therefore, the total activity may be comparable to those of known antioxidants. Furthermore, ingestion of these aroma compounds may help to prevent in vivo oxidative damage such as lipid peroxidation which is associated with cancer, premature aging, atherosclerosis, and diabetes (Lee and Shibamoto 2000: Lee et al. 2000).

Non-nutritive components (antinutritional factors)

Beans contain several components that traditionally have been considered to be antinutrients, such as TIs, phytate (inositol hexaphosphate), oligosaccharides, and saponins (Liener 1989; Siddhuraju et al. 2002; Siddhuraju and Becker 2005). Saponin, PA and polyphenol contents in grains of various varieties of black gram and mung bean amphidiploids ranged from 2746 to 2972, 697 to 750 and 702 to 783 mg/100 g, respectively (Kataria et al. 1989). Tannin contents change with seed colour. TN inhibit the digestibility of protein, whereas PA reduces the bioavailability of some essential minerals (Duhan et al. 1989; van der Poel 1990). The anti-nutritive properties of phytates stem from their ability to chelate calcium, iron, magnesium and zinc. PA (myo-inositol hexaphosphate) is the main seed storage molecule for phosphorous. PA is necessary for normal seed development and germination although its concentration in different bean varieties is variable (Lolas and Markakis 1975). PA and its salts (phytates) represent between 54 and 82% of the phosphorous content of the bean i.e. between 0.5 and 1.6% of the seed weight (Lolas and Markakis 1975). PA chelates various divalent metal ions and is implicated in their reduced absorption leading to deficiency symptoms in animals and humans in diets predominated by legume seed proteins (Sandberg et al. 1993). The catabolism of phytate is controlled by phytase and some other acid phosphatases that allow the phosphorous to be assimilated. Without these enzymes, phytate passes through the intestinal system without being degraded, so contributing to the phosphorus load of the resulting manure (Lott et al. 2000). Although the effect of phytate in reducing mineral bioavailability in plant foods is an important consideration, it has also been postulated that PA may play a role in reducing cancer risk, possibly because of its antioxidant effects (Graf and Eaton 1990; Messina 1999). Specifically, it has been suggested that PA may lower the risk of colon cancer (Harland and Morris 1995) and perhaps breast cancer (Vucenik et al. 1997). Phytate has also been implicated in the reduction of cholesterol and other lipids due to its presence in high fibre diets (Shamsuddin et al. 1997; Reddy 1999; Midorikawa et al. 2001).

More recent information suggests, however, that the antinutrient label may be an oversimplification, especially in the case of oligosaccharides and saponins. TIs from beans can certainly interfere with protein digestion, and in some species of animals do cause pancreatic enlargement and enhance chemically induced pancreatic tumors (Grant 1989). However, boiling dry beans generally reduces the TI content by 80–90% (Duarte-Rayas *et al.* 1992) and there is little reason to think that the amount of TIs obtained by eating commonly consumed beans would exert any adverse effects in humans (Liener 1994; Messina 1999). In contrast to TI, the TI and chymotrypsin inhibitor found in beans, es-

pecially soybeans, has been studied as an anticancer agent (Kennedy and Manzone 1995). Saponins are glycosides composed of a lipid-soluble aglycone that consists of either a sterol or, more commonly, a triterpenoid structure attached to water-soluble sugar residues that differ in their type and amount. The major sources of dietary saponins are legumes, and many types of saponins can be present in the same bean. Saponins are very poorly absorbed. Most saponins form insoluble complexes with 3-β-hydroxysteroids and are known to interact with and form large, mixed micelles with bile acids and cholesterol. Although saponins were shown to lower cholesterol in some animal species, the hypocholesterolemic effects of saponins in humans are more speculative (Milgate and Roberts 1995; Messina 1999). Saponins may have anticancer properties, as suggested by a recent rodent study that found that a saponincontaining diet (3% by wt) inhibited by about two-thirds the development of azoxymethane-induced preneoplastic lesions in the colon (Koratkar and Rao 1997; Messina 1999). However, given that human intake of saponins is generally \leq 200–300 mg/d whereas total food intake is \approx 500 g (dry weight), it is not clear to what extent these results in rodents are relevant to humans (Ridout et al. 1988).

STRATEGIES TO IMPROVE THE PROTEIN QUALITY OF MUNG BEAN

More than 40 years ago, diets containing beans were first shown to markedly increase flatulence (Steggerda and Dimmick 1966). In 1970, it was reported that the oligosaccharides in beans were responsible for gas production (Rackis et al. 1970). The oligosaccharide content of dry beans is $\approx 25-50$ mg/g (Kuo *et al.* 1988; Carlsson *et al.* 1992) and because of the discomfort and social embarrassment associated with flatulence, some people opt to avoid beans entirely. For most of the population, it might seem desirable to remove α -galactosides from pulses by technological or genetic means (Guillon and Champ 2002). Commercial products such as Beano, (AkPharma Inc., Pleasantville, NJ) a digestive aid that contains $\alpha\text{-galactosidase, are}$ available so that individuals can eat beans without discomfort. Additionally, it is possible to remove substantial amounts of oligosaccharides and to markedly reduce flatulence by changing the water in which beans are boiled one or more times (Anderson et al. 1979). However, these nondigestible oligosaccharides have been identified as prebiotic agents (van Loo et al. 1999), i.e. food ingredients potentially beneficial to the health of consumers. There may be some beneficial effects associated with oligosaccharide consumption. The oligosaccharides, because of their growthpromoting effect on bifidobacteria, have been hypothesized to promote the health of the colon, increase longevity, and decrease colon cancer risk (Mitsuoka 1982; Benno et al. 1989; Koo and Rao 1991). Beans are one of the least expensive protein sources for resources poor people and considered as a major source of this important nutrient in many less developed countries. However, protein indigestibility is a common problem with these beans and the utilization of available protein and carbohydrates in legume feedstuffs are much less than that calculated from the chemical composition. This is attributed to the presence of various antinutritional factors (ANFs) such as TI, phytates, saponins, lectins and polyphenolic compounds (Liener 1989; Siddhuraju et al. 2002; Siddhuraju and Becker 2005). Some of these also diminish the bioavailability of trace elements and proteins. Several studies have been reported on the nutritional and antinutritional composition of dry beans from different agro-ecological zones and localities. There is a continued need to carry out this type of work with new varieties that are released principally on the basis of yields, rate of maturity and resistance to disease and draw some over all conclusions taking into account nutritional aspects as well. On the other hand, Shahidi (1997) reported that some antinutrients might exert beneficial health effects at low concentration. Therefore, manipulation of processing conditions may

be required to remove or reduce certain unwanted components. Traditional methods of processing and cooking legumes have been evolved to give acceptable, appetizing and nutritious products. Processing of legumes increases the digestibility and enhances the aroma, sensory qualities and nutritional attributes. Processing not only improves palatability of foods but also increases the bioavailability of nutrients. Attempts to increase the utilization of legumes have employed a wide range of processing techniques such as soaking, boiling, autoclaving, radiation, cooking, roasting, dehulling, germination, fermentation, supplementation with various chemicals and enzymes and recently extrusion cooking (van der Poel 1990; Gujska and Khan 1991; Bishnoi and Khetarpaul 1994; Fernandez et al. 1997; Alonso et al. 1998; Alonso et al. 2000a; Abd El-Hady and Habiba 2003). Extrusion cooking has advantages including versatility, high productivity, low operating costs, energy efficiency and shorter cooking times. Extrusion cooking application to legume processing has developed quickly during the last decade, and can now be considered as a technology of its own right. Legume extrusion cooking would allow reduction of ANFs and therefore improve the nutritional quality at a cost lower than other heating systems (baking, autoclaving, etc.) due to a more efficient use of energy and better process control with greater production capacities (Reimerdes 1990; Alonso et al. 1998; Quintana et al. 1998; Alonso et al. 2000b).

However, none of the aforementioned methods is able to remove completely all the detected antinutrients that are present in feed materials. Hence, a combination of processing methods was generally more effective than a single method (Siddhuraju and Becker 2005). The effects of processing on the removal of undesirable antinutritional compounds using simple and cost-effective processing options for use in developing countries in order to make best use of their nutritional value of the beans are necessary.

Soaking, dehulling, cooking treatments and their combinations

Unfortunately, the culinary and nutritional quality of many bean varieties leave much to be desired. Generally, bean seeds need to be soaked and must be cooked to render them palatable. Soaking is a preliminary step common to almost all methods of preparing legumes, prior to cooking. It helps in the removal of seed coat to shorten the cooking time. Soaking is a simple technological treatment that is often used by mothers to prepare complementary foods at home. Moreover, it can be a simple prolongation of the obligatory washing of the seeds and can also have other advantages, such as facilitating dehulling or swelling of seeds. Soaking, the most common treatment for partial elimination of α galactosides from grains, becomes more efficient when bicarbonate is added, due to the greater long-time soaking (16 h) in bicarbonate solution 0.02-0.07% (w/v) caused remarkable reduction in the antinutritional factors in cowpea (Vijayakumari et al. 2007; Siddhuraju and Becker 2005; Ibrahim et al. 2002). Previous studies have shown that a long soaking period before fermentation or germination, leads to a reduction in phytate content and to an enhancement of mineral HCl-extractability, used to estimate mineral bioavailability (Sandberg and Svanberg 1991; Duhan et al. 2002).

Dehulling of legume seeds and splitting the cotyledons are often carried out for better product profile and acceptability. Dehulling reduces cooking time and it shows a negligible effect on the total protein content and amino acid composition. Dehulling also removes TN that lower protein digestibility (Bressani and Elfas 1980). As seed hulls are rich in fibre and tannin content (Liener 1989) dehulling significantly reduced both crude fibre (Eusebio 1991; Davis *et al.* 2002) and tannin content (Booth *et al.* 2001), and their removal leads to a slight increase in protein content. PA and TIs, however, are concentrated in the cotyledons, so that dehulling did not show any significant influence on ANFs

(Vasagam et al. 2007).

Heat treatment of all kinds inactivates antinutritional enzymes and improves flavor and overall acceptability of the foods prepared. Roasting, parching, toasting and frying are some of the dry heat processing methods used for whole legume seeds. The most common way by which food legumes are processed is by soaking and subjecting them to a thermal process. Although the main reason for thermal processing is to render the grain soft, its effects go beyond the changes in physical structure and texture (Bressani 1985, 1989; Bressani and Sosa 1990; Bressani 1993; reviewed extensively by Pugalenthi and Vadivel 2007 for Mucuna pruriens seeds). Cooking brings about a number of changes in physical characteristics and chemical composition of food legumes. It has been observed, by earlier workers, that different cooking methods improve the nutritional quality of food legumes to various extents (Nielson 1991). Improvement in protein quality of pigeon pea has been reported after the partial removal of polyphenols as a result of a simple boiling method (Singh 1993). Heat processing in general, improves the nutritive value of legume proteins, by inactivating TI and growth inhibitors and haemagglutinins (Swaminathan 1974; Tharanathan and Mahadevamma 2003). Cooking inactivates heat-labile anti-nutritional compounds as well as permits the digestion and assimilation of proteins and starch (Kigel 1999; Broughton et al. 2003). Cooking beans also solubilises the proto-pectin within the middle lamella forming soluble pectin that depolymerises rapidly during heating, allowing water to enter cells of the cotyledon (Stanley and Aguilera 1985). Modifying the composition of the middle lamella may thus render bean seeds easier to cook. Cooking beans by extrusion yields food products that are often equal and sometimes superior in quality to food produced by wet cooking. In the extrusion process the bean particle size, the level of water in beans, the temperature, and the feed rate and velocity of extrusion are important factors to consider in developing products of high nutritive value. On the other hand roasting beans, which enhances their flavor, also reduces their nutritive value by lowering the levels of available lysine (Bressani 1993). Dehulling followed by cooking increases protein quality and digestibility (Bressani et al. 1984; Bressani 1993). This effect is due to the removal of not only crude fiber but also of TN, both of which are present in the hulls of the bean which may contribute to decreased protein digestibility. Heat processing, when done under controlled time and temperature, usually improves the protein quality of food grain legumes (Bressani 1985). Table 1 shows the effect of dehulling, in terms of cooking time at atmospheric pressure, on the protein quality of beans (Bressani et al. 1984). Increased cooking time reduced protein quality in both the whole and dehulled beans, probably due to loss of available lysine.

In mung beans, domestic processing and cooking methods including dehulling, soaking, ordinary and pressure cooking of soaked and unsoaked seeds, and sprouting significantly lowered PA, saponin and polyphenol contents of the amphidiploid seeds (*V. mungo, V. radiata*). Soaking for 18 h removed 31 to 37% of the PA; the extent of removal was higher with long periods of soaking. Saponins and polyphenols were relatively less affected. Loss of the antinutrients was greater when soaked instead of unsoaked seeds were cooked. Pressure cooking had a greater effect

 Table 1 The effect of cooking and dehulling on the protein efficiency ratio (PER) and protein digestibility (PD) of *P. vulgaris*.

Cooking time (min)	Whole bean		Dehulled bean	
	PER	PD (%)	PER	PD (%)
10	0.91	60.8	1.60	70.8
20	0.66	61.6	1.35	73.1
30	0.51	59.9	1.45	71.6
40	0.43	60.3	1.15	73.1
50	0.46	57.7	120	69.7
Casein	2.60	91.2		

Source: Bressani 1984

than ordinary cooking (Kataria *et al.* 1989). Similarly, pressure cooking/autoclaving of raw seeds of various legumes significantly decreased the various antinutrients (Khalil and Mansour 1995; Mubarak 2005; Siddhuraju and Becker 2005). Though the soaking process was less effective in reducing PA and TI it significantly reduced the tannin content of both cow pea and mung bean, in agreement with earlier investigations in different varieties of legume seeds (Vijayakumari *et al.* 1997; Khalil 2006).

Heat treatment is particularly important in the preparation of mung bean for consumption, from the point of view not only of acceptability but also of improvement on protein digestibility. Sensory evaluation of cooked mung bean in terms of taste, colour, aroma and texture has an organoleptic panel indicated that there was no significant difference between mung bean cooked for 25, 30, 35, 40 and 45 min. An *in vitro* protein digestibility (IVPD) assay was used to examine the effect of cooking time on protein digestibility. A maximum improvement in IVPD of 4.83% was obtained by cooking for 38.6 min. No remarkable changes in most amino acids were found between raw and cooked samples except that tryptophan was decreased by 4.69% and a 10.29% loss of threonine occurred with the optimum cooking time (96 \pm 100°C for a time of 38.6min) (Abd El-Moniem 1999).

It has been reported by Rehman and Shah (2004) that, ordinary and microwave cooking reduced the total fiber content of V. mungo but the maximum reduction was observed in a pressure cooker. Similar observations were also made in the case of other dietary fibre components of the food legumes. In order to minimize the losses of dietary fibre, it is suggested that legumes should be cooked, either by the ordinary method or in a microwave oven instead of a pressure cooker (Rehman and Shah 2004). Effects of common processing and cooking methods on sugar and starch contents and starch digestibility (in vitro) of mung bean were investigated by Kataria and Chauhan (1988). Soaking reduced the level of total soluble sugars, reducing sugars, non-reducing sugars and starch and improved starch digestibility, significantly. Cooking (both ordinary and pressure cooking) increased the concentrations of the sugars and digestibility of starch of soaked and unsoaked mung seeds. Starch content, however, decreased. Similar results were obtained by the same investigator in studying the effects of different domestic processing and cooking methods on starch digestibility (in vitro) and protein digestibility (in vitro) of four strains of amphidiploids (black gram × mung bean). An increase of 35 to 48% and 22 to 25% was observed in starch digestibility and protein digestibility, respectively, when the seed of amphidiploids were soaked for 18 h. In another study, the effects of soaking (in water for 16 h) and extrusion conditions including barrel temperature (140°C and 180°C) and feed moisture (18% and 22%) on antinutrients, total and phytate phosphorus and protein digestibility of whole meal of four kinds of legumes (peas, chickpeas, faba and kidney beans) were investigated (Abd El-Hady and Habiba 2003). The results obtained indicated that soaking and extrusion significantly decreased antinutrients such as PA, TN, phenols, α-amylase and TIs. Moreover, extrusion processing decreased the percentage of PA phosphorus to total phosphorus. The IVPD of legume extrudates was also improved. Therefore, extrusion of legumes soaked a priori in water for 16 h is recommended to improve the nutritive value of these legumes in order to increase its utilization by human and animal when consumed directly or as an ingredient of certain meals (Abd El-Hady and Habiba 2003).

Sprouting, germination and blanching

Worldwide, increasing popularity of fresh and fresh-cut produce has been observed together with the consumer perception that these products are healthy, tasty, convenient and fresh (Garrett *et al.* 2003; Orlandi *et al.* 2002; Bari *et al.* 2003). Seed sprouts are among the products that have

gained rapid popularity in the last few decades (Shetty et al. 2003). Sprouts can be easily obtained by germinating the seeds in the dark for up to 4 days. In many countries, mung bean sprouts are one of the most popular vegetables sold in public markets due to availability and nutritional quality. Sprouting does not require soil or solar radiation and is not limited to seasonal growth. Large amounts of sprouts can be obtained in a relatively short time. Sprouts are a cheap source of certain vitamins in the diet and some vitamins are synthesized in the germinating seeds. It is generally known that germination markedly improves the nutritional quality of legumes. Sprouted mung beans contain similar amounts of lipids, protein, starch, polysaccharides other than starch, lignin and ash compared to the raw bean (Rao and Belavady 1978; Jaya and Venkataraman 1981). A dramatic increase in ascorbic acid in legume seeds has been observed during germination. Also, the concentration of a number of other vitamins is high in germinated legume seeds. It is well known that the sprouting process results in an improvement in the vitamin content. Magaram et al. (2006) showed that folacin content on dry weight basis increased several times in alfalfa (Medicago sativa) and mung bean sprouts during germination. Mature alfalfa and mung bean sprouts were found to contain 186 and 178 µg folacin per 100 g fresh weight, respectively.

Germination caused a significant decrease in total protein, fat and carbohydrate contents with increased germination time while non-protein nitrogen, ash and fiber contents were significantly increased. Mineral contents (Na, K, Ca, P, Mg, Fe and Mn) increased during germination. Germination significantly improved *in vitro* protein digestibility. Protein solubility indexes, water absorption and emulsification capacities, foam capacity and foam stability were significantly improved with increase in germination time while fat absorption decreased. The germination of mung beans for 3 to 4 days resulted in a significant increase in the ascorbic acid content from 30 to 54 mg/kg in the seed to between 73 to 383 mg/kg in the sprouts (Fordham *et al.* 1975).

Germination increased protein content of mung seeds, due to the utilization of carbohydrates as an energy source (Liener and Kakade 1980). Evidence of protein synthesis during germination was presented earlier by Kylen and Mccready (1975). They found the protein content of all sprouts was higher than that of the ungerminated seeds. Prudente and Mabesa (1981) showed that the protein quality of mung beans was considerably improved after 60 hours of sprouting. The nutritive value of the sprouts did not appear to be affected by whether the sprouts were grown under dark or illuminated conditions. Noor et al. (1984) observed an improvement in the true digestibility of protein in sprouted mung beans but also observed a small decrease in the protein quality on germination (net protein utilization 28.0 raw to 26.0 in germinated seed). Venkataraman et al. (1976) showed that the true digestibility of the nitrogen of cooked mung beans improved significantly when the seeds were allowed to germinate for 24 or 72 hours. The biological value of the protein was unaffected by germination, but the net protein utilization of the 72-hour germinated seeds was significantly greater than the ungerminated or 24-hour germinated seeds.

Germination resulted in a significant decrease in the ANFs. The levels of TIs and TN decreased compare to the controls. Reduction in PA and hemagglutinin activities increased with increased germination time (Kataria *et al.* 1989). Various attempts have been made to study the effect of germination on the reduction of antiphysiological factors and changes in the organic constituents of legumes in order to improve their nutritive value, but contradictory results are reported in the literature, particularly with respect to protein quality. Most of the reports on the subject indicate that TI activity is retained in germinated food legumes without affecting nutritive value, an observation deserving more research. Likewise, starch is broken down and both flatulence factors and polyphenolic content are reduced (Noor *et al.* 1980; Singh and Jambunathan 1981).

Furthermore, germination is one of the most efficient biological treatments for removing α -galactosides. Sprouted seeds contain less raffinose family oligosaccharides (RFO) as they are used as a source of available energy in the germination process (Rao and Belavady 1978; Jaya and Venkataraman 1981). The reduction of these oligosaccharides during germination leads to a reduction of the flatus potential of the beans caused by the action of intestinal anaerobic microorganisms. Jaya and Venkataraman (1981) noted that as the RFO were degraded during sprouting a significant increase in glucose, galactose and sucrose also occurred. After germination for 48 h at 20°C 40-60% of pea oligosaccharides disappeared (Dostalova et al. 2001). The combined effect of germination and microwave treatment and/or conventional drying further decreased the α -galactoside content of germinated peas (Kadlec et al. 2001). In another study, the effect of mung beans germination on the content of nutrient compounds, antinutritional factors, in vitro digestibility and functional properties was studied by El-Adawy et al. (2003). In another study by Vasagam et al. (2007), mung bean processed with germination in combination with autoclaving obtained higher protein content, lower ANFs and maximum coefficient of total tract apparent digestibility. The significant increase in crude protein content of the cow pea and mung bean on germination can be attributed to production of growth enzymes (Sunday et al. 2001) and consumption of other seed components during the germination process. Similar to these observations, a significant increase in protein content on germinations of seeds have been reported by Uwaegbute et al. (2000) and Rivas-Vega et al. (2006) in cow pea and Mubarak (2005) in mung bean. However, in the study by Vasagam et al. (2007) only slight changes were noticed in crude fibre and crud lipid content, they also reported a significant influence on crude fibre and crud lipid content, which may be due to differences in the germination duration. Otherwise the increase in duration of germination lead to increase in crude fibre content of germinates (Oloyo 2004). Eskin and Wiebe (1983) reported that germination reduced PA content in germinating seed, due to increased phytase activity. After 48 h of germination, tannin contents of mung beans (Barroga et al. 1985) were reduced by 23-36%. Germination of mung beans for 72 hours results in a fall in the PA content from 2.05 g/kg of dry beans to 1.42 g/kg for soaked and germinated beans. Tabekhia and Luh (1980) showed that while the total phosphorus content of the beans remained constant (4.4 g/kg) during germination, the proportion of inorganic phosphorus rose as germination proceeded. Reddy et al. (1978) pointed out that during germination phytase breaks down PA in the seed, yielding a source of phosphorus, cations and inositol to the germinating seeds. It can be assumed that the phosphorus content of germinated seeds is more available than in ungerminated seeds. Germination of mung beans for 48 hours resulted in significant reductions in the phytate and tannin contents with a consequent increase in the ionizable iron content (Rao and Prabhavathi 1982). This increase in iron availability may be quite significant in areas where iron deficiency is widespread and mung beans form a significant portion of the local diet.

Shastry and John (1991) reported that germination increased TI activity in legumes, attributed to TI to the phenolic content in the seeds. Effects of common processing and cooking methods on sugar and starch contents and starch digestibility (in vitro) of mung bean were investigated (Kataria and Chauhan 1988; Kataria et al. 1992). Germination decreased starch thereby raising the level of the soluble sugars. Starch digestibility was increased appreciably. The effects of some traditional processes, such as dehulling, soaking, germination, boiling, autoclaving and microwave cooking, on the nutritional composition and antinutritional factors of mung bean seeds were studied by Mubarak (2005). Germination and cooking processes caused significant decreases in fat, carbohydrate fractions, antinutritional factors and total ash contents. All processes decreased the concentrations of lysine, tryptophan, threonine and sulfurcontaining amino acids. However, all treatments were higher in total aromatic amino acids, leucine, isoleucine and valine contents than the FAO/WHO 1992. Dehulling, soaking and germination processes were less effective than cooking processes in reducing TI, TN and hemagglutinin activity contents. Also, germination was more effective in reducing PA, stachyose and raffinose. Germination resulted in a greater retention of all minerals compared to other processes. IVPD and protein efficiency ratio were improved by all processes. The chemical score and limiting amino acids of mung bean subjected to the various processes varied considerably, depending on the type of process. Also it has been recommended by the author to use microwave and autoclaving cooking for mung bean seed preparation in households and restaurants, not only for improving the nutritional quality, but also for lowering the cooking time (Mubarak 2005).

Blanching has very little effect on amino acid, proteins and lipid contents of sprouted seeds (Farhangi and Valadon 1982). While the amino acid composition of the protein remained reasonably stable on storage, the free amino acids were quickly lost on bottling and storage. The lipids of the processed sprouted seeds remained relatively stable to processing and storage, but there was an increase in simple lipids as complex lipids were slowly degraded. Blanching mung beans has little effect on the total carotenoid content of sprouts (Farhangi and Valadon 1982) but results in a 50% loss of vitamin C. Canning and bottling of sprouts led to considerable losses of vitamin C, while the carotenoid content remained relatively stable. Storage of bottled or canned bean sprouts led to further losses of vitamin C and total carotenoid content.

High hydrostatic pressure process (HHP)

There is increasing worldwide interest in the use of HHP because of the advantages of this technology over other methods of processing and preservation. HHP offers homogeneity of treatment at every point in the product due to the fact the applied pressure is instantaneously and uniformly distributed within the HHP chamber (Mertens and Deplace 1993). Other important advantages are: 1) significant or total inactivation of microorganisms (Hoover *et al.* 1989; Knorr 1993); 2) better functional and nutritional retention of ingredients in the processed products, with improved food quality parameters (Hayashi 1989; Cheftel 1991); and 3) significant energy savings in comparison to thermal stabilization techniques, because once the desired pressure is reached, it can be maintained without the need for further energy input.

Recent studies have demonstrated that under optimal conditions, HHP may inactivate the ANFs of grains while preserving food quality and constituents (Estrada-Giron et al. 2005). However, a vast amount of information regarding the processing of grains by traditional methods is available in the literature; little research concerning the application of HHP in processing of cereal grains and legumes has been reported. There is no literature targeted on the use of HHP and its effect on micronutrients in mung bean and their subproducts; however, it is known that protein, carbohydrate content and micronutrients in legume systems are relatively similar. Thus, one can assume that the effect of HHP on cereal grains and legumes may be similar to what occurs during research conducted on mung bean. During HHP treatment, allergenic proteins from rice grains are solubilized; while no apparent alteration in color, shape, or size of treated seeds occurs at moderate pressure (Kato et al. 2000). Soybeans (tofu) subjected to HHP treatment has been shown to reduce microbial population while increasing protein digestibility. Other constituents of grains such as vitamin A were not significantly affected, while water soluble vitamins (B1, B6, and C) were well retained (85%). Other applications of HHP for cereals include wheat and barley flours, and activity of amylases. A further possibility of creating new textured products from doughs subjected to HHP

was studied as well (Estrada-Giron et al. 2005).

Radiation processing

Radiation processing has emerged as a safe, clean and effective method to disinfect, sterilize and preserve food and agricultural commodities (Nair and Sharma 1994; Farkas 1998). Popularity of irradiated foods will definitely augment among the consumers if adequate information is accomplished about food irradiation, labeling and safety (Bayram and Delince 2004; Gunes and Tekin 2006; Bhat et al. 2007b). It has been observed by earlier workers, that radiation processing improves the nutritional quality of food legumes to various extents. Improvement in protein quality of soybeans and broad beans has been reported after the partial removal of TI and haemaglutinin as a result of a radiation processing simple (Farag 1998). PA, a-amylase inhibitor and oligosaccharids, were inactivated to a considerable extent when legume sample were irradiated (Siddhuraju et al. 2000, 2002). This reduction in PA might be due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation (de Boland et al. 1975)

The effects of irradiation on nutritive characteristics of peas (Pisum satinum L.), cowpeas (Vigna unguiculata L. Walp), lentils (Lens culinaris Med), kidneybeans (Phaseolus vulgaris L.), and chickpeas (Cicer arietinum L.) were examined by Hania and El-Niely (2007). The proximate composition, levels of anti-nutrients (PA, TN), available lysine (AL), IVPD, and protein efficiency ratio (PER) in the growing rat were analysed. The results showed that moisture, crude protein, crude fat, crude fiber, and ash were unchanged by the irradiation. Radiation processing significantly reduced the levels of PA, TN, and AL. IVPD and PER were significantly enhanced in a dose-dependent manner, relative to un-irradiated control samples, for all legumes. The data sets for each legume exhibited high correlation coefficients between radiation dose and PA. TN. AL. IVPD, and PER. These results demonstrated the benefits of irradiation on the nutritional properties of these legumes (Hania and El-Niely 2007). Higher protein digestibility after irradiation treatment may be due to increased accessibility of the protein to enzymatic attack. However, this effect could also be due to inactivation of proteinaceous antinutritional factors (van der Poel 1990). Also, it was reported by Rady et al. (1987) that the nutritional value of all varieties of beans, based on chick growth, was significantly improved by irradiation. Therefore, it could be concluded that the irradiation process offers a good treatment for legumes to reduce or eliminate their ANFs with consequent increase in their digestibility and thereby increase the utilization of their proteins.

Fermentation

Solid state fermentation (SSF) process represents a technological alternative for a great variety of legumes and cereals, or combination of them, to improve their nutritional quality and to obtain edible products with palatable sensorial characteristics. Fermentation is a low-cost and the most economical technique of production and preservation of foods in developing countries. Processing food by fermentation has been practiced by man for centuries and has been used quite extensively in various parts of the world, particularly in the Orient (Khalil 2006). The number of foods that normally undergo fermentation is relatively high, and there are obviously many changes in chemical composition and nutritive value. Food fermentation processes are reliant on both endogenous and microbial enzymatic activities for the degradation of starches, lipids, proteins, antinutritional and toxic factors. Fermented legumes have increasingly gained interest in food applications because of its possible nutritional and health benefits and the potential use as a food flavoring agent. Examples of indigenous food fermentations are mung sauce (shoyu), Japanese miso, Indonesian tempeh,

Indonesian *tape ke ten*, Japanese *sake* (Steinkraus 1983), and Egyptian *kishk* and *bouza* (Morcos *et al.* 1973). Some general observations made by different workers on the effects of fermentation on nutritive value include increases in B_{12} as well as in other vitamins of the B group. Likewise, there are increases in protein quality, increased availability of various nutrients, and removal of antiphysiological factors. Of particular significance is the supplementary effect induced by microbial growth on a substrate (Dworschak 1982).

Fermentation completely removed TI, oligosaccharides and reduced remarkably PA. However, TN noticeably increased in cowpea (Ibrahim et al. 2002). Rhizopus oligosporus is a food-grade fungus that has been widely used in solid-substrate bioconversion systems to produce valueadded food products. 'Tempeh' is one such traditional Indonesian soybean food, which is bioprocessed with different strains of Rhizopus spp. (Hachmeister and Fung 1993; Han et al. 2001). Fermentation with R. oligosporus reduced PA by 30.7% in soybean, 32.6% in cowpea and 29.1% in groundbean (Egounlety and Aworh 2003). Stachyose decreased during fermentation with a reduction of 83.9%, 91.5% and 85.5% respectively for soybean, cowpea and groundbean while raffinose remained fairly constant. Galactose, the predominant sugar, glucose, fructose, maltose and melibiose increased during the first 30 and 36 h of fermentation of cowpea and ground-bean, but decreased thereafter (Egounlety and Aworh 2003). During the solid-state bioconversion (SSB) of mung bean substrate by R. oligosporus, with the goal to enhance health-linked functionality, the α amylase inhibition linked to diabetes management and Helicobacter pylori inhibition linked to peptic ulcer management were investigated in bioprocessed extracts. The protein content and β -glucosidase activity of the substrate which are indicators of effective fungal colonization, increased with growth. The phenolic content increase with growth was linked to fungal β-glucosidase activity, indicating phenolic mobilization (Egounlety and Aworh 2003). The major implication from this research is that SSB is a good strategy to improve the phenolic content of mung beans for enhanced functionality with improved antioxidant activity that contributes to α -amylase inhibition relevant to potential diabetes management and H. pylori inhibition linked to peptic ulcer management (Randhir and Shetty 2007)

Unlike fermented soy foods, which have been extensively studied, no enough studies on fermented mung foods been carried out. Published work on mung bean has focused on their proximate composition and protein availability and on the effects of modifying basic processing parameters such as soaking, dehulling and heat treatment. In another study by Khalil (2006), germination and/or fermentation processes for Egyptian breeds of mung seeds were carried out with three Lactobacillus strains namely, L. reuteri, L. casei, and L. heleviticus. This study gave an insight into the impacts of combined effect of germination and fermentation on the nutrient status and functionality of the mung bean product to improve the nutritional value as well as enhance the antioxidant activity. Fermentation of germinated or ungerminated mung bean with the three Lactobacillus strains resulted in an increase of protein content and in vitro protein digestibility. The increase in solubility and digestibility in fermented seeds could be attributed to the proteolytic activity of Lactobacillus (Fadda et al. 1999). It was postulated that the increase of protein susceptibility to proteolytic enzymes, due to partial protein denaturation and pH during fermentation, leading to increased solubility and digestibility (Czarnecki et al. 1993). However, lactic acid fermentation did not improve protein digestibility of pea seeds, while fermentation and/or extrusion of bean seeds significantly increased protein digestibility (Czarnecka et al. 1998). Kiers and co-workers (2000) reported a tremendous increase in solubility and in vitro digestibility in fermented mung, cowpea and maize with Rhizopus spp. Also, fermentation treatments were effective in decreasing the phytate

content as well as reducing TI activity, while L. casei was the most efficient probiotic culture in reducing those antinutritional factors. The combined treatment of germination and lactic fermentation utilizing Lactobacillus species is recommended process for enhancing the nutritional quality of legume protein (Khalil 2006). Chitra et al. (1996) found that germination and fermentation greatly increased the IVPD and remarkably decreased the total dietary fiber with little effect on calcium, magnesium and iron contents. In another study by Shimelis and Rakshit (2008), dry beans (Phaseolus vulgaris) were also subjected to natural fermentation and then assessed for flatus-producing compounds, antinutrients and in-vitro protein digestibility. Results showed an important decrease in raffinose oligosaccharides, antinutritional components and appreciable improvement in in-vitro protein digestibility. For all varieties of dry beans, raffinose and stachyose (the main α -galactosides in raw bean) concentration reduced prominently to undetectable level and 92-95%; respectively after 96 h of natural fermentation. On the other hand, treatment of cowpea flours for 2 h at 50°C with crude fungal preparations having an α-galactosidase activity equivalent to 64 units μg^{-1} protein, brought about a mean decrease of 82.3% for stachyose and 93.3% for raffinose (Somiari and Balogh 1993).

Complementation effects on dietary protein of mung bean with cereal: possibilities to improve and upgrade nutritional quality of diets

Growth is the most sensitive and readily measured indicator of health and nutrition for the individual. It is also a more general index of health in a community because it is dynamic and reflects positive change. Despite general improvements in food availability and health and social services, hunger and malnutrition exist in some forms in nearly all countries. Between 1975 and 1990 the average prevalence of protein-energy malnutrition (PEM) in children in Africa, Asia, the Middle East, and the Americas combined, as estimated by FAD/WHO, was reduced from 47.5% to 40.8%. Nevertheless, there were 155 million underweight children in Asia in 1990, representing 44% of children under five years of age (FAW/WHO 1992). It has been demonstrated that a diet based on cereal and legumes such as rice, soy beans, and groundnuts or rice, mung beans, and sesame can provide adequate protein, fat, and energy intakes for young children (Tontisirin and Valaiphatchara 1987).

For many centuries, beans have remained part of the human diet in several countries on all continents. Black and mung beans are the commonest variety in Latin America; they are usually consumed as dried mature beans together with rice. The combination of rice and dried mature black beans supplies various nutrients including essential amino acids, folate, soluble fiber, copper, magnesium, iron, potassium, calcium, zinc, and linolenic acid (Bazzano *et al.* 2001; Leterme 2002; Leterme and Munoz 2002: Bazzano *et al.* 2003; Olivares *et al.* 2004). Although there are several varieties of beans that occur in different sizes, shapes, and colors, their nutrient composition is quite similar to that of black beans.

Biological evaluations using rats were carried out to determine the complementation effects on dietary protein of cooked mung bean/rice and cooked germinated mung bean/ rice mixtures by Noor et al. (1984). On an isoproteic basis, mung bean protein was found to be of a lower quality than rice protein. Upon complementation with rice, however, the protein quality of the ungerminated and germinated mung bean/rice mixtures steadily increased when rice was incorporated to provide 25, 50 and 75% of the protein in the diet. A comparison study between germinated and ungerminated mung bean/rice mixtures indicated that the latter mixture was of a better protein quality. Nevertheless, replacement of 75% of the dietary protein of mung bean by rice showed no difference between the germinated and ungerminated mung bean (Noor et al. 1984). Furthermore, in another study in Thailand, four supplementary food mixtures based on cereal

and legumes were developed, with protein contents ranging from 13.2 to 16.5 g/100 g and fat from 10.6 to 13.2 g/100 g, whose energy content meets or exceeds the recommended Thai standards, carried out a project in two villages between 1978 and 1980 (Dhanamitta et al. 1983) in which these mixtures played an essential role. The project was extremely successful, as indicated by a decrease in the prevalence of PEM from 55 to 21% in 18 months. In different countries and cultures, the mung bean is processed into a food product in different ways. The beans are dried, debranned, and ground into flour in India and Pakistan, whereas in China and the United States they are usually allowed to sprout. Certain American producers of Chinese foods either carry out canning of the sprouts alone in water, or mix them with other Chinese vegetables such as water chestnuts. Furthermore, the protein in mung beans is quite deficient in the sulfur amino acids. In China, this deficiency is corrected in part by eating the beans or their sprouts with cereal products such as rice, or with small amounts of eggs, fish, meat, or poultry. The other protein sources balance the amino acid deficiencies in the legume. A dietary supplement are comprised of two major components, viz. dehydrated mung bean sprout and oat fiber was introduced by Joseph Kovacs in a US Patent Issued in 1996 (US patent No 53408334). Ratios of components can vary but typically they are present in an amount of 1 to 1.5 parts of dehydrated mung sprout to 1 part of oat fiber. In addition to the two major components, a variety of fillers, flavoring agents, binders, minerals and vitamins as well as typical adjuvants used in the art can be used. Sorbitol as a sweetener can be mentioned as well as dicalcium phosphate and magnesium stearate as mineral agents are also suitable. Mung beans, together with β -glucan enriched oat-groat. The resulting powdered composition can be used as a dietary supplement having a positive effect on mammalian health. In one aspect of the invention, a novel method is provided for producing one of the plant origin components. Furthermore, the present dietary supplement relates to a method for retarding the loss of muscle tissue for patients who are dieting and losing weight (Kovacs 1996).

Furthermore, legume-fortified weaning foods are of good nutritive value and have been shown to prevent protein-energy malnutrition (Cheryan et al. 1979; Egounlety et al. 2002). Fermentation of grain legumes before its incorporation or the co-fermentation of both cereals and grain legumes has the advantage of solubilizing the material constituents as well as reducing or eliminating the antinutritional factors (Zamora and Fields 1979; Egounlety and Syarief 1992). Whole maize meal or dehydrated fermented maize (ogi) flour fortified with soybean, cowpea or groundbean tempe, were studied by Egounlety (2002) and Egounlety et al. (2002) for production of high protein-energy legume-fortified weaning foods. In this study, Tempe-fortified maize-based weaning foods had high protein quality and can support the growth of infants in developing countries especially during the critical weaning period (6-12 months).

CONCLUSIONS

Despite considerable progress in recent decades, the world still falls short of the goal of adequate food and nutrition for all. No other people feel this more than children living in the developing countries. Another problem is that most of the traditional first supplementary foods introduced in many countries are prepared from cereal and legumes, commonly mixed. Supplementary food mixtures based on cereal and legumes should be improved because they arc limited in certain macronutrients and micronutrients such as calcium and phosphorus, and perhaps some vitamins and trace elements. In addition, antinutritive factors in legumes may reduce the bioavailability and absorption of some nutrients; fortunately, most of these factors can be significantly reduced by many processes. Although mung beans are consumed by millions of people, little research has been carried out on improving their nutritional value. Priority should be given to screening lines for high essential amino acid content, particularly methionine. Strains should be identified that contain lower levels of antinutritive factors and selections should be made to reduce their flatus potential. Alternatively, proper appropriate processing techniques should be applied to mung bean in order to enhance its nutritional value. The nutritional profile of beans shows that they have much to offer; beans are high in protein, low in saturated fat, and high in complex carbohydrates and fiber. Beans are also a good source of several micronutrients and phytochemicals. Mung beans (*Phaseolus aureus*, *Vigna radiatus*) are thought to be beneficial as an antidiabetic, low glycaemic index food, rich in antioxidants. An increased consumption of mung bean sprouts, particularly by people consuming western-type diets, could have a significant effect on cardiovascular disease, which is a major problem for these people. The use of legumes assumes significance as a cheap and concentrated source of proteins, due to the high cost of proteins of animal origin and their inaccessibility by the poorer section of the population. An increased consumption of plant seeds would provide a more economical way to feed people than via the animal industry. Evidence suggests that these sources of legumes such as mung beans may provide health benefits when included in the daily diet. Inactivation and/or removal of undesirable components are very essential in improving the nutritional quality and organoleptic acceptability of beans and in turn help to effectively utilize their potential as human food and animal feed. Hence, more studies are required to affirm the positive effect of the combination of processing methods which are generally more effective than a single method. For large populations, a simple and inexpensive processing technique which changes the seed composition of beans and improves its acceptability is necessary.

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