L-Dopa (L-3,4-Dihydroxyphenylalanine):
A Non-Protein Toxic Amino Acid in *Mucuna pruriens* Seeds

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ABSTRACT

The seeds of *Mucuna pruriens*, a potential tropical under-utilized legume, were reported to contain high level of protein (26-30%), desirable amino acid, fatty acid and mineral composition with good nutritional properties, comparable with that of other common legumes. It is consumed as food by people living in the rural areas of India, Sri Lanka, Philippines, Nigeria, Ghana, Brazil, Malawi and other countries. L-Dopa (L-3,4-Dihyroxyphenylalanine), a non-protein phenolic amino acid, extracted from *M. pruriens* seeds is being used in the treatment of Parkinson's disease. Clinical studies also proved the effectiveness of L-Dopa extracted from *M. pruriens* seeds in curing Parkinson's disease over chemically synthesized L-Dopa. But, on the other hand, the pharmaceutically active factor, L-Dopa, is potentially toxic and an antinutrient compound in a nutritional point of view. It is reported to inhibit the digestibility of protein and starch in the diet and thereby reduced the growth performance of experimental animals. It has been reported to cause some serious gastrointestinal disturbances such as nausea, vomiting and anorexia and also induce favism, if consumed by humans or animals. However, in contrast, its antioxidant potential was evidenced by certain studies. Even so, many researchers are working with L-Dopa the world over in either a nutritional or clinical aspect, and there is much conflicting information on its antinutritional and toxic effects on growing animals as well as its pharmacological side effects. However there exists no summative scientific assessment to date. Hence, the present review has been emphasized to evaluate the available information regarding the L-Dopa content of *M. pruriens* seeds.

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INTRODUCTION

One of the serious problems facing most developing countries is the scarcity of good quality protein for the teeming human population and dwindling livestock industry. Although the common legume seeds have been playing a key role as an economic protein source throughout the world, their production is not enough to meet the increasing protein requirements (Carlini and Udedibie 1997). Further, stiff competition exists between the increasing human popula-

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tion and expanding livestock industry for consumption of legume seeds as a protein source, leading to an inadequate supply of protein, which ultimately results in protein-energy-malnutrition in many developing countries, including India. Hence, there is an urgent need to explore other alternative, additional or novel protein foods for the world of tomorrow. In this context, recent research trends have been directed to identify and evaluate under-utilized legume seeds, whose potential remains untapped to the extent that they deserve, in view of the pressure on conventional legumes which arises due to increasing multiple uses (Janardhanan *et al.* 2003a).

Among the various under-utilized legumes, velvet bean [*Mucuna pruriens* var. *utilis* (Wall ex Wight) Baker ex Burck], a tropical legume having good nutritional properties, merits a wider use as a food legume in South Asian countries (Pugalenthi *et al.* 2005). Velvet bean belongs to the family Fabaceae and is widely distributed in Southern and Southeastern Asian regions and in some parts it is cultivated as a green manure and cover crop (Buckles 1995). This little-known legume has a wider adaptation and exhibits more tolerance to many adverse environmental conditions such as drought, low soil fertility, high soil acidity and wide range of climatic conditions (Duke 1981; Labo Burle *et al.* 1992; Buckles 1995; Carsky *et al.* 1998; Carsky *et al.* 2001).

This annual climbing herb has pinnately trifoliate leaves with purple or white coloured flowers and is self-pollinated (**Fig. 1**). Pods are long and linear with pubescent velvety hairs, thus acquires the name velvet bean, and seeds are uniseriate with white or black coloured or mottled seed coat (Pugalenthi *et al.* 2005). The plant exhibits better growth under warm, moist conditions at an altitude of below 1600 m. The life cycle of this plant varies from 152-155 days and flowering takes place on the 59th to 66th day. It has many favourable agro-botanical characters such as a high fertility index, number of pods/cluster, number of seeds/pod, etc.,
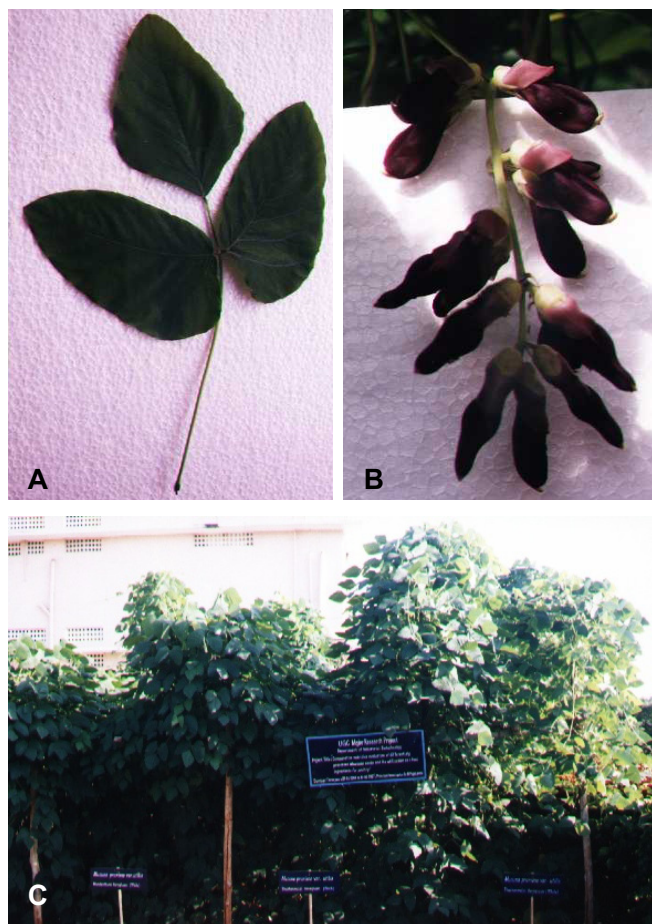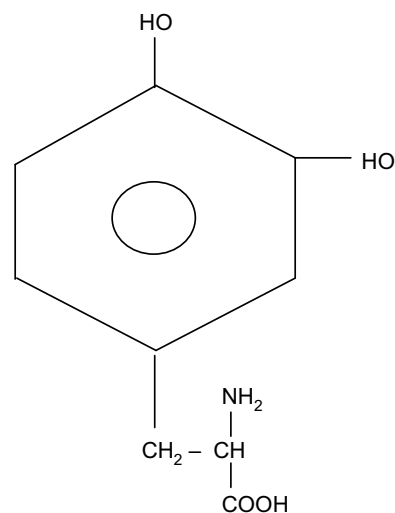

Fig. 2 Chemical structure of L-Dopa (L-3,4-Dihydroxyphenylalanine).

and exhibits high yield potential (2.9-6.9 t/ha), thus, it is considered to be one of the most productive legumes of the world (Vadivel and Janardhanan 1997; Gurumoorthi *et al.* 2003; Pugalenthi and Vadivel 2006).

The beneficial impact of velvet bean on the agricultural sector has been documented in a number of studies. It suppresses weeds through physical smothering and probably also through a certain degree of allelopathy (Gliessman 1983; Fujii *et al.* 1992; Sahid *et al.* 1993; Carsky *et al.* 1998). It has been found to control some weeds such as Cogongrass (*Imperata cylindrica*) better than herbicides (Udensi *et al.* 1999). L-Dopa (L-3,4-dihydroxyphenylalanine; **Fig. 2**), a non-protein amino acid, has conferred on *Mucuna* seeds some level of resistance to bruchid beetles (Bell and Janzen 1971; Rehr *et al.* 1973) and its presence in seeds resists attack from insects and biological infestations during storage (Vadivel and Janardhanan 2001). *Mucuna* has been reported to exhibit disease resistance most especially to *Macrophomina phaseolina* (Berner *et al.* 1992) though it is known to be vulnerable to several diseases (Duke 1981; Thurston 1997). *Mucuna* has been found to be resistant to some nematodes and it is effective in lowering the nematode populuation for the subsequent crops (Reddy *et al.* 1986; Mashkoor *et al.* 1990; Kloepper *et al.* 1991; Rodriguez-Kabana *et al.* 1992; Nogueira *et al.* 1996; Queneherve *et al.* 1998; Carsky *et al.* 1998).

The long-term effect of *M. pruriens* as a cover crop on the communities macrofauna and nematofauna under maize cultivation in Southern Benin was studied by Blanchart *et al.* (2006). The soil under *Mucuna* cover crop shows higher macrofauna density (especially termites, earthworms, millipedes, centipedes) and biomass (especially earthworms and termites), higher density of facultative phytophagous, bacterial-feeding and predatory nematodes, and lower density of obligatory phytophagous (*Criconemella*, *Scutellonema* and *Meloidogyne*) nematodes. The modification of the composition and activity of soil biota under *Mucuna* might partly explain the potential of *Mucuna* for soil restoration. The benefits of velvet bean as natural fertilizers in maize production in Uganda was studied by Kaizzi *et al.* (2006).

The seeds of velvet bean have been used in indigenous ayurvedic medicine as a uterine stimulant (Jayaweera 1981; Lorenzetti *et al.* 1998), powerful aphrodisiac, emetic (Amin *et al.* 1996; Tripathi and Upadhyay 2001) and to treat nervous disorders and arthritis and also have been used against snakebite in traditional medicine in India and West Africa (Houghton and Skari 1994; Aguiyi *et al.* 1999; Guerranti *et al.* 2001). Gastroprotective actions of L-Dopa was studied by Mercer *et al.* (1998). The effect of L-Dopa on indome-

Fig. 1 *Mucuna pruriens.* (**A**) Leaf material of velvet bean plant. (**B**) Flowers of velvet bean plant. (**C**) Velvet bean plant in the field.

thacin-induced intestinal ulceration in rat was studied by Kiro *et al*. (1992). The results demonstrated that L-Dopa has a protective effect on indomethacin-induced small bowel injury in rat. The hypoglycemic activity of velvet bean with relation to anti-diabetic potential was demonstrated by Grover *et al*. (2002). A mild antimicrobial activity of L-Dopa was reported by Zhao *et al*. (2007). L-Dopa is used to provide symptomatic relief in Parkinson's disease (Siddhuraju *et al*. 2000; Pugalenthi *et al*. 2005).

Velvet bean seeds have been reported to contain a high level of protein (26-30%) and starch (34-40%), desirable amino acid, fatty acid and mineral composition with good nutritional properties (Siddhuraju *et al*. 2000; Vadivel and Janardhanan 2001; Vijayakumari *et al*. 2002; Siddhuraju and Becker 2003a; Siddhuraju and Becker 2005; Pugalenthi *et al*. 2005; Vadivel and Pugalenthi 2007) and their nutritional quality is comparable with that of soybean and other common legumes in relative nutrient profiles (Siddhuraju and Becker 2003a). Velvet bean seeds have been traditionally used as food by peoples living in rural areas of India, Sri Lanka, Philippines, Nigeria, Ghana, Brazil and Malawi and other countries. In India, certain ethnic groups, particularly, the Northeastern tribes and Kanikkars tribes in Kerala State and Dravidian tribals in Tamil Nadu State consume this legume, after boiling, either as such or mixed with other cereals (Janardhanan *et al*. 2003b; Pugalenthi *et al*. 2005). The seeds are used as a coffee substitute in Guatemala and Southern Mexico and earn the name Nescafe. Successful incorporation of velvet bean seed flour as a protein ingredient at 15% level in the production of high protein biscuits was also reported (Ezeagu *et al*. 2002). Velvet bean leaves are used as fodder and the seed meal is used as cattle feed along with cotton seed meal. Some experiments have also been conducted on its utilization as a dietary protein source in animal feeds (del Carmen *et al*. 1999; Muinga *et al*. 2003; Siddhuraju and Becker 2003a; Perez-Hernandez *et al*. 2003; Pugalenthi *et al*. 2005).

However, although it constitutes a potential source of protein and other nutrients, their utilization as food or feed remains limited due to the presence of certain antinutritional compounds like total free phenolics, tannins, L-Dopa, phytic acid, lectins, oligosaccharides, protease inhibitors and α-amylase inhibitors (Siddhuraju *et al*. 2000; Siddhuraju and Becker 2001a; Vadivel and Janardhanan 2001; Vijayakumari *et al*. 2002; Janardhanan *et al*. 2003b; Vadivel and Pugalenthi 2006, 2007). Raw seeds were reported to cause vomiting and diarrhea when large amounts are ingested by human beings and other monogastric animals (Duke 1981; Afolabi *et al*. 1985) and their presence in the diets have been reported to reduce the growth rates of broiler chicks (del Carmen *et al*. 1999) and egg production in laying hens (Harms *et al*. 1961) and also reduce the growth performance of fishes (Siddhuraju and Becker 2003a).

Among the various antinutritional compounds in velvet bean seeds, L-Dopa is considered as a major antinutrient and proved to be potentially toxic (Siddhuraju and Becker 2002). The oxidized products of L-Dopa bind with SH compounds (cysteine) of proteins and form protein-bound 5-S-Cysteinyldopa cross-links, which leads to the polymerization of proteins and reduces the protein digestibility (Takasaki and Kawakishi 1997). It also has been proven to be toxic in individuals with glucose-6-phosphate dehydrogenase deficiency in their erythrocytes, resulting in induced favism (Nachama and Edward 1967).

The available information on L-Dopa often shows confusing and conflicting data concerning its antinutritional and medicinal properties. There are different opinions among researchers with respect to the toxicity of this amino acid, because, even though it is proved to be toxic and antinutritious, evidenced by a fish feeding experiment (Siddhuraju and Becker 2002), the antioxidant property of L-Dopa was also reported (Siddhuraju and Becker 2003b). Although the effectiveness of L-Dopa in the treatment of Parkinson's disease – a progressing disabling disorder associated with a deficiency of dopamine in the brain – over the chemically

synthesized L-Dopa was proved (Hussain and Manyam 1997; Valdes *et al*. 2004; Sayyed and Sudalai 2004), the treated patients often reported to be severely affected by some serious side effects such as hallucinations in addition to gastrointestinal disturbances such as nausea, vomiting and anorexia (Reynolds 1989; Infante *et al*. 1990). Dopamine replacement therapy with L-Dopa remains the standard pharmacotherapy for Parkinson's disease. But unfortunately, chronic L-Dopa treatment is accompanied by development of motor fluctuations and L-Dopa induced dyskinesia (Eskow *et al*. 2007).

Thus, the lack of consensus in the scientific literature on L-Dopa is scattered and conflicting information on the antinutritional and pharmacological properties of L-Dopa is observed. Additionally, in the available literature there is apparent disagreement with regarding to L-Dopa content that varies depending on geographic origin (Laurent *et al*. 2002; Capo-chichi *et al*. 2003; Janardhanan *et al*. 2003b), the particular species and part of the plant examined (Prakash and Tewari 1999; Szabo and Tebbett 2002), the time of harvest (Prakash and Tewari 1999), and climatic considerations (Lorenzetti *et al*. 1998), all of which add to further confusion. Even though many researchers working with L-Dopa throughout the world in both a nutritional and medicinal context, there is no scientific summary of this information to date. Hence, the present review emphasizes and evaluates the information available concerning the toxicology and pharmacology of L-Dopa.

L-DOPA: THE NON-PROTEIN TOXIC AMINO ACID

The toxic non-protein amino acids are not the normal components of proteins but occur in a free state in many plants, particularly in the Leguminosae. These kinds of amino acids are usually concentrated in seeds but can also be found throughout the whole plant and exhibits toxic effects on the animals consuming them and thus limit the utilization of legume seeds as food or feed. Consequently, all these toxic amino acids should be regarded as possessing the potential to exhibit deleterious effects in animals and their toxicity is determined to a significant extent by complex interactions with the nutritionally important amino acids. Often the toxic action of these non-protein amino acids affects the nervous system but a wide range of other effects have also been reported (D'Mello *et al*. 1991). Some examples of such non-protein amino acids are L-canavanine, indospicine, homoarginine, mimosine, β-cyanoalanine, β-*N*-oxalyl-α-β-diaminopropionic acid, α,γ-diaminobutyric acid, djenkolic acid, among others (D'Mello *et al*.1991).

L-Dopa was first isolated over 90 years ago from the fruits of *Vicia faba* (Brain 1976). Since then its occurrence has been noted in a number of other plants, including cereals like wheat and oats, although it is confined to leguminous species (Barbeau and McDowell 1970; Duffus and Duffus 1991). Its presence in plants remained of academic significance until the discovery that the oral administration of L-Dopa relieves Parkinson's disease by Arvid Carission in 1950 (Iversen and Iversen 2007). The widespread application of this therapy created a demand for large quantities of L-Dopa at an economic price level and this has led to the reinvestigation of plant resources. Some important commercial suppliers of L-Dopa are Sigma-Aldrich, Alfaaesar, Abcr, AcrosOrganics, Alkemi, Chemicon, Advtechind, dayangchem, Eurasia, lobachem and Hallopharma. 5 g of L-Dopa costs about 20$ in Himedia and 27.9$ in Sigma-Aldrich chemicals (2007 prices).

Among the various plants, the seeds of the genus *Mucuna* have been found to contain the highest levels of L-Dopa, but even the levels found in this species necessitate the processing of large quantities of material, a difficulty, which is accentuated by the high dosage rate of the drug. The relatively large and consistent L-Dopa concentration in *Mucuna* beans has been well verified. Daxenbichler *et al* (1971, 1972) surveyed a total of 1062 plant species in 160 families and determined that only *Mucuna*'s L-Dopa content

Table 1 L-Dopa content in velvet beans and other leguminous seeds.

Name of legume seed	L-Dopa content of the velvet bean seeds (g L-Dopa/100 g seed flour)	Reference
Mucuna pruriens var. *utilis*	3.62- 6.98	Siddhuraju *et al.* 2000; Vadivel and Janardhanan 2000; Siddhuraju and Becker 2001a, 2001b, 2001c; Diallo *et al.* 2002; Szabo and Tebbett 2002; Myhrman 2002; Siddhuraju and Becker 2003, 2005; Vadivel and Janardhanan 2005; Vadivel and Pugalenthi 2006
Mucuna pruriens var. *pruriens*	4.08- 9.1	Mary Josephine and Janardhanan 1992; Del Carmen *et al.* 1999; Carew *et al.* 2003; Adebowali *et al.* 2005; Vadivel and Janardhanan 2005
Mucuna atropurpurea	2.6	Mohan and Janardhanan 1991
Mucuna gigantean	1.5	Rajaram and Janardhanan 1991
Mucuna cochinchinensis	6.1- 6.2	Diallo *et al.* 2000; Myhrman 2002; Adebowali *et al.* 2005
Mucuna rajada	5.35	Adebowali *et al.* 2005
Mucuna veracruz	6.35- 7.12	Adebowali *et al.* 2005
Mucuna deeringeana	3.87	Adebowali *et al.* 2005
Canavalia ensiformis	2.64	Vadivel and Janardhanan 2005
Canavalia gladiata	2.83	Vadivel and Janardhanan 2005
Cassia floribunda	1.57	Vadivel and Janardhanan 2005
Cassia obtusifolia	1.34	Vadivel and Janardhanan 2005
Mucuna monosperma	4.52- 4.56	Arulmozhi and Janardhanan 1992

was sufficient to merit extraction for medicinal purposes. In this survey, *Mucuna* seed L-Dopa concentration varied between 3.1% and 6.7%. Similarly, Bell and Janzen (1971) measured L-Dopa content in six *Mucuna* accessions collected from different locations and found that the L-Dopa concentration ranged between 5.9 and 9.0%.

Several studies have focused on L-Dopa quantification in plant parts of various *Mucuna* species. In India, the L-Dopa content of *Mucuna* species have reported as much as 9% in some *Mucuna* seeds (Janardhanan and Lakshmanan 1985; Mary Josephine and Janardhanan 1992; Mohan and Janardhanan 1995; Siddhuraju *et al*. 1996), and as little as 1.5% in the seed of *M. gigantea*, which grows wildly in southern India (Rajaram and Janardhanan 1991) (**Table 1**). Pugalenthi *et al*. (2003) reported that the L-Dopa content of two different accessions of *M.monosperma* falls between 4.16 and 4.24%. The L-Dopa content of other under-utilized legumes such as *Tamarindus indica* (2.64%), *Erythrina indica* (2.96%) and *Sesbania bispinosa* (2.01%) was also reported by Pugalenthi *et al*. (2004). The L-Dopa content of velvet bean seeds collected from five different agro-ecological regions of South India was investigated to evaluate the effect of agroclimatic conditions of different growing locations on the L-Dopa concentration in the velvet bean seeds by Janardhanan *et al*. (2003b) and their findings revealed that the existence of diversity in the L-Dopa content among the different accessions ranged from 5.60 to 6.56% (**Table 2**).

There has been considerable confusion with respect to the presence of L-Dopa in the seed coat of *Mucuna*. Indeed, the previous literature has contained assurances that removing the seed coat from velvet beans will eliminate a significant fraction of L-Dopa (Siddhuraju and Becker 2001a). To test that hypothesis, the content of L-Dopa periodically assayed in the seed coats of *Mucuna* samples and has always obtained very low numbers (less than 0.1%). Furthermore, since the seed coat represents only about 10% of the total mass of the seed, the fraction of the total L-Dopa in the coat is negligible. The study conducted with whole vel-

vet beans seeds noted that L-Dopa level fell between 3.63 and 4.7% but that the dehulled seeds were found to contain a higher content of L-Dopa (10-15% higher) than the whole seeds. It clearly indicates that this compound is concentrated largely (more than 90%) in cotyledons and only trace levels in the seed coats (Siddhuraju *et al*. 2000).

Apart from the seed materials, various plant parts of *Mucuna* have also investigated for L-Dopa content, which revealed that L-Dopa largely accumulated in the seeds. Prakash and Tewari (1999) measured L-Dopa concentrations in velvet bean seeds and various parts of the plant collected from different climatic zones of India, which ranged between 0.17-0.35% in leaves, 0.19-0.31% in stems, and 0.12-0.16% in roots, which is similar to the results obtained by Szabo and Tebbett (2002). In another study, L-Dopa was present most notably in the seeds (4.93-5.39%) with the stems and roots containing the next highest levels (0.49 and 0.27%, respectively). Lower than roots, no difference in concentration was found among pods and leaves (0.15%) of velvet beans growing in Benin and Gainesville samples (<0.3% in stems, <0.2% in leaves) (Szabo and Tebbett 2002).

Evidences suggested the existence of high variability in L-Dopa concentration among various accessions of velvet beans collected from different places. The L-Dopa content in velvet bean seeds ranged from 2.2 to 7.2% in a survey of 36 accessions collected from India, Benin, Honduras, Mexico and Georgia, USA (Lorenzetti *et al*. 1998). On the basis of their results, they reported that the *Mucuna* plants growing with in 10° of the equator contained significantly higher levels of L-Dopa than the plants growing far away from the equator regions. Laurent *et al*. (2002) also examined the L-Dopa concentration in seeds of 38 accessions of *M. pruriens* and two accessions of *M. brachycarpa* using High Performance Liquid Chromatography (HPLC). L-Dopa concentration as the percentage of dry weight of seeds varied from a low of 1.81% for an accession named *M. pruriens* var. *utilis* grown in the USA to a high of 7.64% for an accession named *M. pruriens* var. *cochinchinensis* grown in Bénin. Twenty-four accessions *of M. pruriens* out of 38 had a percentage L-Dopa of between 4 and 6%. One of the two accessions of *M. brachycarpa* had a percentage of L-Dopa lower than 4% while the other was between 4 and 6% dry weight. The stability of velvet beans across all the environments indicated that all the accessions may be widely adapted for L-Dopa production. Since many species of *Mucuna* have been shown to produce L-Dopa, its presence and quantification could eventually be used as an interesting systematic biochemical marker.

A wide variation in the L-Dopa content among the *Mucuna* germplasm have been reported (Daxenbichler *et al*. 1972; Mary Josephine and Janardhanan 1992; Krishnamur-

Table 2 Variability in the L-Dopa content of velvet beans growing at different places (based on Laurent *et al.* 2002).

Location name	Latitude	L-Dopa content (g/100 g seed flour)
Hawaii	20°03'	2.26
Honduras	15°07'	4.34
Benin	6°40'	7.64
Florida	26°39'	3.39
Israel	32°08'	5.65
Colombia	3°40'	4.52
Georgia	33°25'	4.71

thy *et al*. 1996; Lorenzetti *et al*. 1998; Qi *et al*. 1999; Laurent *et al*. 2002). Specifically, there is little information on the impact of genotype and environment on the production of L-Dopa, as only two studies to date have addressed this topic (Lorenzetti *et al*. 1998; Laurent *et al*. 2002). Both studies found that both environmental factors and genotype have a major impact on L-Dopa production in *Mucuna* seeds. Among the various environmental factors, both studies have been particularly stressed the impact of latitude, where the plant was grown.

Lorenzetti *et al*. (1998) reported that the variability in L-Dopa content of velvet beans was caused by both environmental effect and genetic nature based on their findings of presence of more L-Dopa in the velvet bean seeds growing near the equator (within 10°) than the plants cultivated far away from equatorial regions (**Table 2**). Among the various environmental factors, they believed that only latitude plays an important role in determining the L-Dopa level in velvet beans. On the basis of their results, they suggested that L-Dopa synthesis is higher in plants grown at low latitudes, near the equator. They also hypothesized, based on the laboratory work done by Pras *et al*. (1993) and Liu and McClure (1995), that variation in the intensity of light and in backscattered ultraviolet radiation, both generally higher near the equator, may be among factors explaining why L-Dopa content was found to be higher at lower latitudes.

Similarly, Laurent *et al*. (2002) found a slight impact of latitude and concluded that other factors also influenced the determination of L-Dopa concentration. These past studies that explored the role of environment and genotype on L-Dopa production in velvet bean seeds were available at different locations worldwide. Thus, the effect of location was confounded by genotype. Wichers *et al*. (1989) also showed that various environmental parameters (e.g., nature of nitrogen source and presence/absence of illumination) affected the production of L-Dopa in cell suspension cultures of *M. pruriens*. This is consistent with the fact that previous studies on L-Dopa synthesis is a function of illumination, which either showed stimulation (Wichers *et al*. 1983, 1989) or inhibition (Brain 1976; Pras *et al*. 1993) of L-Dopa synthesis.

However, the L-Dopa concentration was weakly correlated with latitude at place of growth (Laurent *et al*. 2002), suggesting that latitude-related factors might partly influence L-Dopa synthesis, but other factors might also be implicated. The regression analysis of L-Dopa in seeds against latitude was not statistically significant for any accession. Increasing latitude did not result in statistically higher or lower L-Dopa concentration for any of the accessions. This may suggest that variation in latitude is not an important factor influencing L-Dopa concentration in *Mucuna* seeds, although the results should be interpreted with caution since the sites also varied in many other environmental factors and management.

The relationship of early maturity with L-Dopa content was analyzed by Temple and Huyck (2002). At Colombia, USA, *Mucuna* synthesized the highest L-Dopa in the seeds (6.4%), which is a late matured variety and at all other sites, *M. pruriens* produced the lowest L-Dopa, which may be due to the fact that they mature earlier. If the role of L-Dopa in the plant is to protect it from insects, then an early maturing variety would not need as much L-Dopa because it would be more likely to escape insect pressure. Alternatively, lower L-Dopa in *Mucuna* may occur for some other reason and low L-Dopa may not even be linked with maturity. Prakash and Tewari (1999) reported that the level of L-Dopa of three Indian *Mucuna* species was ranged between 3.6 and 4.2% and they also found that even the half mature seeds of *Mucuna* beans have been reported to have a higher L-Dopa content than the matured dry seeds and therefore it could be determined that the maturity as well as harvesting period of the *Mucuna* beans also influences the L-Dopa content, similar to faba bean seeds (Burbano *et al*. 1995). In addition, it has also been reported that L-Dopa concentrations in *Mucuna* seeds vary with the stage of development (Prakash and

Tewari 1999). This suggests that there is a technology involving genetic expression for L-Dopa synthesis, that can be turned "on" or "off" at specific stages of plant development and seeds of *Mucuna*. This technology, known as Late Embryonic Abundant (LEA) manipulation, carries the potential to control L-Dopa production in tissues as diverse as nodules, guard cells, and seeds (Temple and Huyck 2002).

Fava bean sprouts are a rich source of L-Dopa, the precursor of dopamine and is used in the treatment of Parkinson's disease. Its phytopharmaceutical value was improved by priming the seeds with natural elicitors like fish protein hydrolysates (FPH), lactoferrin (LF) and oregano extract (OE). The elicitors in general stimulated the phenylpropanoid pathway through the pentose phosphate and shikimate pathway and enhanced the production of phenolics. Among the different FPH elicitor concentrations, 2 ml/l elicited the highest phenolic content of 3.4 mg/g on day 2, which is three times higher than that of control (Randhir *et al*. 2002). For all elicitors and control the L-Dopa content was high on day 1 and steadily declined with germination. The L-Dopa content in fava bean elicited by LF showed a 40% increase, where as FPH and OE showed a 20% increase compared to that of the control. The maximal stimulation of L-Dopa content was seen on day 2 for fava bean treated with 2 ml/l of FPH, which was 100% higher than that of control.

Shetty *et al*. (2002) observed the stimulation of nutraceutically relevant phenolic metabolites when germinating fava beans are exposed to ultra violet (UV) and the potential role of the proline-linked pentose phosphate pathway (PPP). Studies have shown phenolic phytochemicals can be stimulated with UV light. The objective was to over-express the PPP through the regulation of the key enzyme, glucose-6-phosphate dehydrogenase (G6PDH), for enhanced phenolics and L-Dopa production and investigate the link to proline metabolism. A total of 15 h UV treatment stimulated L-Dopa on day 1, which reduced over next 8 days of germination. This novel approach provides a mechanism to understand and enhance biosynthesis of important phenolic compounds in plant and legume systems for use in nutraceutical applications. The increase in the co-factors such as NADP/NADPH would result in the over-expression of the phenylproponoid pathway required for the L-Dopa synthesis via phospho-pentose-pathway through the activity of glucose-6-phosphate dehydrogenase (Shetty *et al*. 2002). Kawazoe *et al*. (2007) reported that D-Dopa, the sterioisomer of L-Dopa was oxidized by D-Amino Acid Oxidase and converted into dopamine by an alternative biosynthetic pathway.

On the other hand, many studies reported the levels of apparent genetic variability in L-Dopa content explains the genotypic variation leading to variations in L-Dopa production in velvet beans (Daxenbichler *et al*. 1972; Krishnamurthy *et al*. 1996; Lorenzetti *et al*. 1998, 2002; Capo-Chichi *et al*. 2003). It must be noted that although the studies appear to be heterogeneous in terms of methodologies used, description of materials tested, plant parts examined, age/stage of the tissues, and the geographic location from which seeds were obtained, this final point is very important, because while some studies have clearly shown an interaction of L-Dopa level in the seed with the environmental conditions under which *Mucuna* was grown, the studies themselves do not document those conditions.

The previous literature gives a clear picture of cause and effect in terms of the genetic and environmental factors controlling the presence, absence, and levels of L-Dopa. Due to the central role of L-Dopa in limiting the utilization of *Mucuna* as a food and feed, a formal genotype × environment study was conducted, in which all varieties were grown at all sites. Four to eight accessions were grown in numerous sites in latitudes ranging from 18°S to 30°N (Capo-chichi *et al*. 2003). The results showed that out of the total variance, 42.6-49.1% was attributed to genotype effect, and that environment and genotype by environment effects were either small or not highly significant. Many other environmental and management factors could impact the production of L-Dopa in *Mucuna* seed but their role has been

poorly studied. In view of the earlier reports, one of the major issues needed to be addressed in order to better understand the factors that influence L-Dopa production in the seeds of *Mucuna* species is an exhaustive "genotype × environment" study to assess the effect of each factor on L-Dopa production, as well as on other growth parameters.

Few studies had assessed the role of environment and genotype on L-Dopa production in *Mucuna* plants. To examine the impact of genotype and environmental factors on the L-Dopa concentration the same genotype was grown in a multitude of environments (Capo-Chichi *et al.* 2003). The results of the investigation indicated that the L-Dopa concentration of each accession in each tested environment is due to a mixture of environment main effect (E), genotype main effect (G), and genotype × environment interaction (GE) Genotype had the greater influence on L-Dopa content, accountings for 44.6-49.1% of the total variance of L-Dopa at different sites. The genotype × environment interaction effect appeared minimal when compared with the genotype/accession main effect.

In one study, the combined analysis of variance including both years, genotypes differed significantly in mean L-Dopa concentration in *Mucuna* seeds (Capo-Chichi *et al.* 2003). All accessions may be widely adapted for L-Dopa production because of their stability in L-Dopa production across all environments. The velvet beans were grown in different agroclimatic zones and the growing sites and years were not significantly different. One first order interaction (G × S) and the second order interaction (G × S × Y) were not significant for L-Dopa concentration, while the other first order interactions (G × Y) and (S × Y) were significant at the 5% probability level. The slight or non-significance of these interactions relative to the strong genotype main effect indicates that not too much emphasis should be placed on these interactions and that accessions/genotypes concentration for L-Dopa in seeds was not only dependent upon the environment. These interactions indicated substantial differences in genotype response for L-Dopa concentration in seeds in different environments. The slight variation due to the interactions could also be attributable to site-related factors.

An investigation to study the production of L-Dopa in velvet bean seeds in different places at two subsequent years (2000 and 2001) was carried out by Capo-chichi *et al.* (2003). In 2000, L-Dopa concentration in *Mucuna*'s seeds varied from 3.3 to 5.9%. In 2001, L-Dopa concentration varied from 2.4 to 6.4%. In Zimbabwe, L-Dopa concentrations were similar in both the years, whereas, in Bénin, Florida, and Honduras, L-Dopa concentration in seeds was slightly higher in 2000 than in 2001. The exception was in Belle Glade, Florida where, *Mucuna* tends to synthesize more L-Dopa and in Bénin where levels were similar in both years. This difference may be explained by the fact that seeds for the two-year experiments were obtained from different seed. Thus, there may be seed-to-seed variability for L-Dopa within the same accession and the climatic factors between the two years may also be partly responsible for such variation.

Production of L-Dopa by using tyrosinase enzyme in batch and packed bed reactors was investigated by Ates *et al.* (2007). Tyrosinase has been immobilized by entrapment method in copper-alginate gel. L-Dopa concentration obtained from batch reactor for free and immobilized enzyme was 9.5 and 4.5 mg/l, respectively. L-Dopa concentration was obtained as 1.2 mg/l in the packed bed reactor. When air was introduced L-Dopa production increased 6.4 times in packed bed reactor and the productivity was calculated as 110 mg/l/h. The enzymatic production of L-Dopa using *Erwinia herbicola* cells involves the action of tyrosine phenol-lyase (Tpl, EC 4.1.99.2). Since Tpl is only synthesized under L-tyrosine-induced conditions, the addition of L-tyrosine to the medium is unavoidable when preparing cells (the enzyme source), but severely impedes the pure preparation of the final product L-Dopa. We circumvented this problem by using recombinant *E. herbicola* cells carrying a mutant

transcriptional regulator TyrR, which is capable of activeting the *tpl* promoter in the absence of L-tyrosine (Koyanagi *et al.* 2005).

Production of L-Dopa from tyrosinase immobilized on nylon membrane in a batch reactor was investigated by Pialis and Saville (1998). Since Tpl is only synthesized under L-tyrosine-induced conditions, the addition of L-tyrosine to the medium is unavoidable when preparing cells (the enzyme source), but severely impedes the pure preparation of the final product L-Dopa. We circumvented this problem by using recombinant *E. herbicola* cells carrying a mutant transcriptional regulator TyrR, which is capable of activating the *tpl* promoter in the absence of L-tyrosine (Koyanagi *et al.* 2005).

The capacity of computational multiple steady states in a complex biochemical reaction networks involves in the production of L-Dopa using L-Tyrosine as a substrate and tyrosinase enzyme as a biocatalyst operating in an isothermal Continuous Stirred Tank Reactor (CSTR) was studied by Ho *et al.* (2005). Innovative strategies for the operation of mist trickling reactors for enhancing the L-Dopa production was studied by Huang *et al.* (2004). Intermittent feeding of nutrient medium in the later half of cultivation promoted the productivity of L-Dopa, especially with a misting cycle of 1 h on and 1 h off. Due to the shortage of a nitrogen source during the later stage of cultivation, fed-batch supplementation of B5 medium was initiated after continuous nutrient feeding. The results showed that fairly high L-Dopa productivity was attained (0.644 g/l). The effect of nitrogen source on the production of L-Dopa in a mist trickling reactor was analyzed by Huang and Chou (2006). Although inconsistent results were observed between the shake flasks and mist trickling reactor for L-Dopa content, reproducible effects of replenishment of NH_4^+ and sucrose from flask to mist trickling reactor were obtained.

Biosynthesis of L-Dopa containing proteins by human cells was studied by Rodgers *et al.* (2004). Many cell proteins contained L-Dopa and seemed to be synthesized as their full-length forms. The cellular removal of L-Dopa-containing proteins by THP-1 cells was by proteolysis involving both the proteasomal and the lysosomal systems. The rate of cellular proteolysis of L-Dopa-containing proteins increased at lower levels of L-Dopa incorporation but decreased at higher levels of L-Dopa incorporation. *In vitro* production of L-Dopa by genetically modified primary rat fibroblast was studied by Leff *et al.* (1998). The effects of aeration rate, agitation rate and the impeller type on the L-Dopa production by *S. hassjoo* cells were investigated by Huang *et al.* (2002) in a 5 liter agitated bioreactor. A dual impeller system consisting of a flat-blade turbine and pitched-blade turbine provided a high oxygen transfer rate that was beneficial to the cell growth and L-Dopa production. At 0.06 vvm and 300 rpm, the dual impeller system supported high cell growth rate and L-Dopa content with an average growth rate and growth index similar to shake flask production.

Efficient production of L-Dopa by *Stizolobium hassjoo* cell culture was attempted by Huang and Chen (1998) by reformulating Murashige and Skoog (MS; 1962) medium to one more suitable for L-Dopa production. The concentrations of the carbon and nitrogen sources, inorganic nutrients, trace elements and organic supplements in the basal MS medium were optimized for L-Dopa production. Concentrations of phosphate, Ca^{++} and Zn^{++} corresponding to 20, 10 and 0% of the levels present in MS medium maximized L-Dopa content. Also, elimination of the organic supplements and some inorganic compounds enhanced L-Dopa production. Validation of the medium in shake flasks resulted in 2.1–2.3 fold increases in L-Dopa content compared to the control medium.

L-Dopa production by *Stizolobium hassjoo* cells was investigated by Huang *et al.* (1995). The favorable conditions for obtaining high productivity of L-Dopa were found to be the precise control of pH, adequate IAA (indole-3-acetic acid) concentration and color of cells which reflect melanin

production. By referring to the assumption that the prosthetic group of the phenol oxidase is copper and that the catalytic activity of the enzyme is based on the change of the valency of cupric ions, Cu^{++} concentration in culture medium was increased. When Cu^{++} concentration in basal MS me-dium was increased 25-fold (0.625 mg/l of $CuSO_4 \cdot 5H_2O$), the total L-Dopa content in the culture medium was about two-fold more than obtained in basal MS medium. A similar effect was confirmed with Co^{++}. Another strategy used in this work was to lower the ionic strength in the culture medium so that inhibition of the cell growth can be reduced. Dilution of the MS medium influenced the L-Dopa content in the cells. A 1/3 dilution of MS medium supplemented with optimized concentrations of constituents determined from this investigation resulted in the 2.05-fold increase in L-Dopa content.

As an alternative approach to the production of L-Dopa from a cheap raw material, Park et al. (1998) constructed a hybrid pathway consisting of toluene dioxygenase, toluene cis-glycol dehydrogenase, and tyrosine phenol-lyase. In this pathway, catechol is formed from benzene through the sequential action of toluene dioxygenase and toluene cis-glycol dehydrogenase, and L-Dopa is synthesized from the resulting catechol in the presence of pyruvate and ammonia by tyrosine phenol-lyase cloned from Citrobacter freundii. When the hybrid pathway was expressed in E. coli, production of L-Dopa was as low as 3 mM in 4 h due to the toxic effect of benzene on the cells. In order to reduce lysis of cells, Pseudomonas aeruginosa was employed as an alternative, which resulted in accumulation of about 14 mM L-Dopa in 9 h, showing a stronger resistance to benzene.

A heterogenous asymmetric catalysis was developed by Valdes et al. (2004) to produce L-Dopa via cinchonine mediated pd/c asymmetric reduction of the corresponding cinnamic acid precursor. Reaction proceeded well giving rise to good optical yield (44%). The use of O,O'-dibenzyl groups in the substrate appears to be the key to this optical purity and deserves further investigation. A simple and effective procedure for the enantio selective synthesis of L-Dopa was described by employing the sharpless asymmetric dihydroxylation as a key step to introduce chirality was studied by Sayyed and Sudalai (2004). William S. Knowles was awarded with a Nobel prize in 2001 in Chemistry for his work on chirally catalyzed hydrogenation reactions, which are used in the synthesis of L-Dopa.

ANALYTICAL METHODS

Since the compound L-Dopa received more attention in both nutritional and medicinal fields, many researchers were involved in the analysis of L-Dopa. The need to out-source a simple and accurate technique for L-Dopa analysis originates from the fact that such an analysis is often difficult to conduct and requires expensive equipment. Due to the long distances and coordination logistics involved, sending samples away is slow, cumbersome and expensive, especially for those conducting research in developing countries and when a large number of samples are involved. While developing and testing processing methods is important, rough estimates of L-Dopa concentration are often more efficient than exact measurements which involve long waits. In addition, certain samples, such as animal tissues and milk, are either extremely difficult or impossible to send abroad for analysis due to import restrictions. Preferably, most L-Dopa analysis should be conducted on-site in the country. Due to these problems, scientists all over the world have attempted to develop some relatively quick, more sensitive and easy methods to determine the L-Dopa concentration that would rely on inexpensive and widely available equipment.

In earlier stage, while assays for L-Dopa in plant material had been described (Daxenbichler et al. 1971, 1972), researchers working with L-Dopa typically had neither the laboratory facilities nor the technical training to run the tests. Furthermore, while these assays were suitable for screening studies, in which significant amounts of L-Dopa

were being measured, they were not sensitive enough to monitor the progress of detoxification procedures, in which the desired end result is a vanishing concentration of an antinutritional factor. Hence, several studies have focused on the development of a suitable method for the quantification of L-Dopa in plant parts of various species of Mucuna (Bell et al. 1971; Daxenbichler et al. 1972; Brain 1976; Wichers et al. 1983, 1989; Lorenzetti et al. 1998; Myhrman 2002).

Previous methods for the analysis of L-Dopa have involved a non-specific colorimetric test (Arnow 1937; Maggi and Cometti 1972; Szent-kiralyi 1979). Later a method for separation of the compound using column chromatography followed by quantification using UV-Spectrophotometry (Daxenbichler et al. 1972) was developed and the recently developed methods of estimation through amino acid analyzer (Bell and Janzen 1971; Prakash and Tewari 1999) were time-consuming and specific only for L-Dopa, whereas the method developed by Marquardt and Frohlich (1981) is more specific for vicine, convicine and their hydrolytic products rather than L-Dopa.

The methodology described by Daxenbichler et al. (1971) was widely used for the measurement of L-Dopa concentration, in which, the seed flour was extracted with 0.1 N HCl and heated in boiling water for 5 min. After cooling, an equal volume of ethanol was added and the mixture was shaken mechanically for 10 min, centrifuged at 2000 rpm for 10 min and the supernatant was retained. The residue was re-extracted with an equal volume of ethanol and the supernatants were pooled and made up to a known volume with ethanol. Quantitative estimation of L-Dopa concentration of the supernatant was measured under UV light (283 nm) after correction for background absorption.

Earlier L-Dopa measurements relied on spectrophotometry, but today, most researchers prefer liquid chromatography, which is more exact and finely separates L-Dopa from other compounds with similar light-absorbing characteristics. During the recent efforts Dr. R. Myhrman from Judson College, Illinois, and his students developed a more sensitive method to quantify L-Dopa concentrations for researchers and development workers worldwide (Myhrman 2002). In this procedure, the instrument separates L-Dopa from all other substances in the sample before determining its concentration. This approach results in a much greater sensitivity than possibly with more traditional methods and enables accurate measurements even at low L-Dopa concentrations in the sample.

Ranjith et al. (2007) used a Liquid Chromatography-Mass Spectrophotometer for the analysis of L-Dopa. A capillary electrophoresis coupled with indirect chemiluminescence detection method was developed for the determination of L-Dopa by He et al. (2006). A HPLC method was developed to detect the L-Dopa and its metabolites (dopamine, dihydroxyphenylacetic acid and homovanillic acid) following the oral administration of L-Dopa or its prodrugs by Cannazza et al. (2005). Schmelli et al. (1993) developed an HPLC method using electrochemical detection, which is rapid and simple method to detect the intracellular levels of L-Dopa. A new method of spectrophotometric determination of L-Dopa was developed by Hasan et al. (1995). By this method, L-Dopa can be determined with a limit of detection of 52 nanogram/ml relative standard deviation of 0.2% for three replicate measurements of a solution containing 4 µg/ml.

An Oxovanadium-Salen complex (N,N-ethylene-bis (sali-cylideneiminato) Oxovanadium) thin film deposited in a graphite-polyurethane electrode was investigated by Teixeira et al. (2007) with regard to potential use for the detection of L-Dopa in flow injection system. Application of a gold screen-printed electrod, an electrochemical sensor for monitoring the L-Dopa level in stationary solution as well as flow system was studied by Bergamini et al. (2005). This method was successfully applied for the determination of L-Dopa in commercial dosage forms without any pretreatment. An automated procedure for the photochemical determina-

tion of L-Dopa has been developed by Perez-Ruiz *et al.* (1993). This method allows the flourimetric determination of L-Dopa in the range of 1.5-12.7 µg/ml with a sampler frequency of 35 samples/h.

A sensitive and selective method for the voltammetric determination of L-Dopa in pharmaceutical formulations was developed by Teixeira *et al.* (2004) using a carbon paste electrode modified with trinuclear ruthenium ammine complex $[(NH_3)_5Ru^{III}–O–Ru^{IV}(NH_3)_4–O–Ru^{III}(NH_3)_5]^{6+}$ (Ru-red) incorporated in NaY zeolite. The results obtained for L-Dopa in pharmaceutical formulations (tablet) was in agreement with compared official method. In conclusion, this study has illustrated that the proposed electrode modified with Ru-red incorporated zeolite is suitable valuable for selective measurements of L-Dopa. A new cyclo-dextrin-modified micellar electronic chromatographic method for the enantiomeric separation of L-Dopa has been developed by Shen and Zhao (2004). This method can be employed for optical purity analysis of L-Dopa drug and allowed the determination of presence of 0.14% D-Dopa in L-Dopa drug with well peak identification.

Recently, a simple and specific procedure for the quantification of L-Dopa in *Mucuna* seeds was developed by Siddhuraju and Becker (2001b) by using reversed-phase HPLC, but it is still not cheap for developing countries. This method is capable of detecting L-Dopa at as low as 0.1 µg/ml and analysis time is also considerably less, i.e., less than 8 min for elution of this compound and no pre-treatment is required. In this method L-Dopa was extracted from finely-ground and defatted seed flour and mixed with 0.1 N HCl for 10 min at room temperature and then homogenized in the cold; subsequently it was kept on a magnetic stirrer for 1 h at room temperature. The mixture was centrifuged at 13,000 rpm for 15 min and the supernatant was collected. The extraction procedure was repeated twice and the supernatants were pooled together and filtered through a 0.2 µm glass filter. The chromatogram consisted of a Merck-Hitachi model L-7100 HPLC pump, an L-7450 UV detector and photodiode array detector, an L-7200 autosampler with injector valve containing a 100 µl sample loop, a D-7000 interphase module and an LC organizer. The analytical column was reverse phase C18 and the absorbance was monitored at 282 nm and peak heights and areas were monitored. Two solvents used were the eluting solvent, consisting of water, methanol and phosphoric acid in the ratio of 975.5:19.5:1 and the washing solvent (70% methanol). Separation was performed at room temperature (22°C) and the flow rate was 1.2 ml/min. The agronomic and medical research community were much benefited from the availability of such a sensitive and reliable technique for the assay for L-Dopa.

ANTINUTRITIONAL AND TOXIC EFFECTS OF L-DOPA

L-Dopa is considered as a major antinutritional compound in velvet bean seeds (Siddhuraju *et al.* 2000; Siddhuraju and Becker 2001a; Vadivel and Janardhanan 2001; Vijayakumari *et al.* 2002; Janardhanan *et al.* 2003b; Pugalenthi *et al.* 2005; Vadivel and Pugalenthi 2006, 2007). It is pharmaceutically active and used in the treatment of Parkinson's disease, but potentially toxic in nutritional point of view (Siddhuraju *et al.* 2000). It has been reported to cause gastrointestinal disturbances such as nausea, vomiting and anorexia and it has also been shown to be toxic in individuals with glucose-6-phosphate dehydrogenase deficiency in their erythrocytes and induce favism (Siddhuraju and Becker 2001a). Oxidation products of L-Dopa conjugate with SH compounds (cystein) of proteins to form a protein bound 5-S-cysteinyldopa cross-link and leads to polymerization of proteins and resulted in lowering of protein digestibility (Takasaki and Kawakishi 1997).

Consumption of improperly boiled seeds of velvet bean is known to cause an increase in body temperature and skin eruptions in the consuming tribal peoples, particularly, the

Northeastern tribes and Kanikkars tribes in Kerala, India (Pugalenthi *et al.* 2005). These symptoms were attributed to the presence of L-Dopa in velvet beans (Janardhanan *et al.* 2003b). Evidence suggesting that L-Dopa suppresses the absorption of amino acids has been presented by several authors (D'Mello *et al.* 1991) while Roe (1985) demonstrated that L-Dopa only depressed the absorption of L-phenylalanine in humans.

The high proportion of phenolic substances present in food/feed have been shown to have adverse effects on monogastrics and other warm-blooded animals and it also reduced the feed intake, growth and nutrient availability and increased the endogenous losses of nitrogen through faeces (Makker and Becker 1998). The major portion (80%) of total phenolics present in velvet beans is constituted by L-Dopa. These compounds were readily oxidizable in alkaline pH and high temperatures with moisture and formed a dark coloured compound (Siddhuraju *et al.* 2000). Oxidation of such *O*-diphenolics from *O*-quinones generated unstable and highly reactive molecules that subsequently reacted with other *O*-quinones, amino acids or proteins, evolving into brown, black or red heterogeneous polymers responsible for reduction of proteolytic digestion of food proteins (Zenin and Park 1978; Hurrel and Finot 1984; Rohn *et al.* 2001). The complex formation by oxidized products of L-Dopa with peptide sulphur amino acids reduces the bioavailability of cystein and methionine in the diets.

Monogastric animals clearly experience a number of negative impacts from ingestion of raw *Mucuna* beans having a high L-Dopa content (del Carmen *et al.* 1999; Siddhuraju and Becker 2003a). Evidence presented by Carew *et al.* (2003) pointed out that such effects are only partly attributable to L-Dopa. Topps and Oliver (1993) stated that pigs and poultry suffered acute vomiting and diarrhea on account of the L-Dopa content. In chickens it inhibits their growth and reduces egg production (del Carmen *et al.* 1999; Carew *et al.* 2003). Likewise, pigs lost weight or made little gain (Flores *et al.* 2002).

Inclusion of any level of raw velvet bean seeds (from 10-30%) resulted in progressive reduction in the growth rate with an extreme depression produced by 30% level (del Carmen *et al.* 1999). A large part of the negative effect of the velvet beans on growth performance of poultry birds is undoubtedly explained by the presence of the toxic principles, especially L-Dopa. To remove these toxic constituents, the beans were roasted and fed to Peterson x Hubbard commercial type broiler birds. However, the various levels of heated velvet beans (10, 20 and 30%) in the poultry diet also continued to depress growth except that at the end of the experiment at 42 d of age, broilers which had been fed the 10% level grew as well as controls. This suggests that heating reduced the L-Dopa content to some extent and allowed market-weight broilers to tolerate the 10% level. Other measures such as feed intake, feed conversion and final carcass weights were also better in broilers fed heated velvet beans compared to raw seeds. They analyzed velvet beans for both antitrypsin factor and L-Dopa. Heating destroyed the antitrypsin factor but had no effect on L-Dopa. Therefore, the improvement in broiler performance when velvet beans were heated can be partially explained by a reduction in antitrypsin factor but not by a change in L-Dopa level. Birds fed the velvet bean diet also had the most depressed weight gain (WG) and protein efficiency ratio (PER) and the poorest feed conversion ratio (FCR). The poor performance with diet containing raw *Mucuna* in all other parameters is not only attributable to depressed feed intake but also to the effect of the anti-nutritional factors, including L-Dopa (Ukachukwu and Obioha 1997; del Carmen *et al.* 1999; Bressani 2002; Carew *et al.* 2002; Flores *et al.* 2002).

Levels of 0, 1, 2, 3 and 5% of pure L-Dopa was substituted into the diet at the expense of sucrose in the poultry diet by Carew *et al.* (2002) and they found that L-Dopa caused graded reductions in growth rate and feed intake as their dietary levels increased. Feeding L-Dopa caused little

changes in the anatomy and blood chemistry of chicks than the raw velvet beans. Weights of the pancreas and gizzard increased in chicks fed velvet beans while liver and proventricular weights were unchanged. Lengths of both the small intestine and ceaca also increased with velvet beans. But with L-Dopa all organ weights and lengths diminished as the dietary level increased. Blood plasma levels of triiodothyronine, cholesterol and creatinine decreased with velvet beans in the diet while alanine aminotransferase increased, but with L-Dopa the only change in blood chemistry was a decrease in plasma creatinine. From the study, they concluded that the changes in organ size and blood chemistry in chicks might be partly due to the presence of L-Dopa content of the velvet beans.

All levels of added dietary L-Dopa caused graded reductions in growth and feed intake of poultry birds for the one-week experimental period (Carew *et al*. 2002). Percent reductions in weight gain and feed intake with 1, 2, 3, and 5% L-Dopa were 18, 29, 53, 70, and 12, 31, 67, 83, respectively. Also 0.5, 1.0 and 1.5% levels of L-Dopa caused 8, 23, and 49% reductions in body weight, and 9, 27, and 51% reductions in feed intake, respectively. Weights of heart, liver, proventriculus and gizzard decreased while the weight of the pancreas was unchanged. Lengths of the small and large intestine and caeca also decreased with 1.0 and 1.5% L-Dopa. Of all the chemicals measured in blood plasma, only creatinine decreased and the dietary L-Dopa did not significantly change the others. It should also be noted that during the first 10 days of the experiment, several chicks receiving 1.5% L-Dopa showed aggressive behavior such as feather and toe picking. There was also evidence of pica as demonstrated by eating of paper that lined the floor of the pens. After ten days to two weeks these effects subsided. These results evidenced the possible toxic attributes of L-Dopa on the poultry birds. The work by Carew *et al*. (1998a, 1998b) clearly demonstrated that the L-Dopa ingested at high levels does reduce appetite and growth of chickens.

Pure L-Dopa powder added to a diet may have a different effect than the same substance present as an integral part of the velvet beans (Carew *et al*. 2002). Differences were observed, however, between the effects of velvet bean and L-Dopa on organ growth. Consumption of raw velvet bean caused an increase in the weights of the gizzard and pancreas in spite of the fact that the chicks grew more slowly. Liver and proventricular weights were unchanged while heart weights decreased. But these effects are not a consequence of the L-Dopa in the velvet bean is demonstrated by the fact that no level of dietary L-Dopa caused similar effects. In general, as was expected, birds fed L-Dopa grew more slowly and had decreased weights of heart, liver, proventriculus and gizzard. Also, pancreatic weights were unchanged with L-Dopa, whereas they had increased in chicks fed velvet bean. Another curious observation is the fact that the length of both the small intestine and caeca in chicks fed with raw velvet bean increased even though they grew at a much slower rate. These changes are not due to the L-Dopa content of the velvet bean, which is demonstrated by the observation that lengths of the small intestine and caeca as well as the large intestine actually decreased, as the level of dietary L-Dopa increased.

When analyzing the blood chemistry, the Plasma triiodothyronine (T3) but not thyroxine decreased significantly with 20% velvet bean. Slow growth rate itself can depress plasma levels of T3 (Keagy *et al*. 1987), but Carew *et al*. (2002) observed with L-Dopa that lower plasma T3 levels did not accompany depressions in growth rate. Plasma creatinine was the only blood component that was altered by both raw velvet bean and L-Dopa in the diet. Low plasma creatinine often signals an increase in glomerular filtration rate of the kidney and these results may suggest that consumption of raw velvet bean affects kidney function perhaps through the L-Dopa content (Finco 1997). The enzyme, alanine aminotransferase (ALT), increased in chicks fed with raw velvet bean but not in chicks fed with L-Dopa. Therefore, another factor in velvet beans apart from L-Dopa

apparently causes either hepatic or muscular damage (Lumeij 1997). This observation, coupled with the decrease in plasma creatinine, may argue more strongly for the occurrence of muscle damage rather than liver damage, which might be due to the effect of L-Dopa along with some other antinutrients present in the velvet bean seeds. Although L-Dopa has as dramatic and negative effect on chick growth and feed intake as raw velvet bean, most of the other physiological and anatomical effects of feeding raw velvet bean, except for a decrease in plasma creatinine, did not occur when pure L-Dopa was added to chick diets (Valverde *et al*. 2000). To what extent the continued presence of L-Dopa, or other potentially toxic factors in the heated beans explains the persistently depressive effect of the velvet beans on broiler performance is unknown.

Earlier, Miller (1929) analysed the toxic effect of L-Dopa and hypothesized that it may have played a role in indigestibility and amino acid deficiency in pigs. L-Dopa was found in small amounts in pigs fed a *Mucuna* diet in the first seven (out of nine) sections of the small intestine but in no part of the large intestine. Small amounts of L-Dopa were probably oxidized in the alkaline small intestine and the product unabsorbed, as shown by dark faeces. There was ample evidence that L-Dopa is passed in the urine, which in *Mucuna*-fed pigs turned brownish red and then black quickly and its specific gravity, percentage of solids and percentage of total nitrogen were higher than from pigs with ordinary rations.

L-Dopa in feeds resulted in reduced intake and depressed weight gains of growing pigs. Results for biochemical analysis on blood collected from the pigs fed with velvet beans containing L-Dopa shown that the hematocrit was found to be at a normal level but the GPT (Glutamic-Pyruvic Transaminase), AST (Aspartate Amino Transferase) and bilirubin were severely altered in relation to control animals and were out of the normal range for healthy pigs (Flores *et al*. 2002). All these parameters are metabolically related to the detoxification processes carried out by the liver, and they strongly indicate the toxic effect of L-Dopa and other antinutritional compounds in the blood of the animals whose livers could not properly eliminate them. Macroscopic observations on the livers of pigs fed *Mucuna* confirmed that acute toxic hepatitis had occurred in all pigs, with the central lobules showing a red color and congestion with blood. The periphery of the liver had yellow coloration, with cellular tumefaction and an advanced degree of necrosis. All these symptoms are described in cases of acute toxic hepatitis by Jones and Hunt (1983). These effects may partly be attributed to the toxic effect of high content of L-Dopa in *Mucuna* seeds. Camara *et al*. (2003) estimated the daily L-Dopa intake was 15, 10, 11 and 13 g for toasted, cooked, soaked and raw *Mucuna* treatments, respectively. Among the differently treated velvet beans, toased seeds included diet exhibits better body weight gain (244 g/day) than the cooked (30 g/day) and soaked (6 g/day) seed included diets. In fact, the L-Dopa level in toasted *Mucuna* bean was slightly higher (15 g/day/pig) than the cooked seeds (10 g/day/pig) but yielded the better animal performance could indicate that L-Dopa is not a factor involved in the low feed intake of these diets.

The effect of L-Dopa on the growth performance, metabolic rates and feed nutrient utilization of common carp (*Cyprinus carpio* L.) was analysed by Siddhuraju and Becker (2002). When present at a higher inclusion rate greater, i.e. >7 g/kg diet, it was reported to reduced the growth performance such as body weight gain (BWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER), metabolic rates, and feed nutrient utilization in common carp fishes (**Table 3**). The poor growth performance in fishes fed with diet containing raw *Mucuna* seed meal might be due to unavailability of sulphur amino acids and other essential amino acids in the feed caused by L-Dopa. Increasing the concentration of L-Dopa in the fish diets also significantly reduced the apparent net protein utilization (ANPU) and protein produc-

Table 3 Growth performance and feed utilization of common carp fed with diet containing various levels of L-Dopa (from Siddhuraju and Becker 2002).

L-Dopa content in the diet (g/100g)	Body Weight Gain (BWG) (g)	Specific Growth Rate (SGR)	Metabolic Growth Rate (MGR)	Feed Conversion Ratio (FCR)	Protein Efficiency Ratio (PER)	Apparent Net Protein Utilization (%)	Energy Retension (%)
0	60.7	3.06	15.4	0.93	2.62	42.0	29.0
2.5	58.4	3.01	15.0	0.96	2.52	40.2	26.3
7.0	56.3	2.96	14.7	1.00	2.42	37.4	26.4
14.0	43.1	2.56	12.5	1.16	2.05	31.2	23.0
28.0	23.8	1.81	8.5	1.77	1.33	20.8	15.4
56.0	12.8	1.20	5.4	2.82	0.83	11.7	7.7

tive value (PPV).

This study also demonstrated that the carp fed diets containing L-Dopa were unable to utilize feed energy as efficiently resulting in significantly higher Apparently Utilizable Energy (AUE) portion (Siddhuraju and Becker 2002). The higher AUE and Energy Retension (ER) of these fish clearly demonstrate the effect of high L-Dopa levels on these parameters. The energy expenditure in these fish remained high and comparable with control fish even while absorption of nutrients and growth was significantly lower. These fish also had higher oxygen consumption and metabolic rate compared to control fish indicating a stress that could have been caused by high dietary levels of L-Dopa. The higher oxygen consumption per unit body mass gain and the tendency for having higher metabolic rates of fish that consumed the diet containing L-Dopa showed that these fish have metabolic stress. However, the growth and metabolic rate of the treatment groups were similar initially. This might be due to the L-Dopa absorbed during this period, which possibly resulted in inducing high metabolic rate and inability to use feed energy resulting in a significantly lower AUE value in the fish fed diets containing L-Dopa at the end of the experiment. Interestingly, no significant variation was observed between the control group and the fish fed different levels of L-Dopa with respect to body composition such as moisture, protein, ash and the energy value. Nonetheless, the correlation co-efficient of determination between body mass gain and concentration of L-Dopa offered to fish clearly indicated a dose-dependent negative effect of L-Dopa (Siddhuraju and Becker 2002).

The increasing inclusion of >6 g/kg of L-Dopa in the diet significantly reduced the growth rate of fishes and the diet with the highest level (24 g/kg) of L-Dopa gave the poorest performance for final body mass, BWG, SGR, FCR and PER in carp (*Cyprinus carpio* L.) (Siddhuraju and Becker 2001c). The deposition of absorbed nutrients was not affected except at the highest concentration. L-Dopa obviously interfered with the digestion and absorption of nutrients. Only the highest inclusion level of L-Dopa (56 g/kg) produced significantly lower levels of carcass lipid and energy content. On the other hand, L-Dopa insignificantly increased the cholesterol levels in plasma compared with the control group and it was also evident that L-Dopa had a less pronounced effect on lipid rather than protein metabolism. Siddhuraju and Becker (2002) concluded that the reduced growth performance of fish fed diets containing L-Dopa might be related to the interference of L-Dopa and its oxidized products with amino acid utilization.

Although unpleasant side effects have not been noted in ruminants such as cows (Burgos *et al.* 2002; Muinga *et al.* 2003) and goats (Castillo-Caamal *et al.* 2003; Matenga *et al.* 2003; Mendoza-Castillo *et al.* 2003; Perez-Hernandez *et al.* 2003) with the consumption of *Mucuna* beans, since L-Dopa is rapidly eliminated from the animals body (Moffat 1986; Dollery 1999), it is unlikely that L-Dopa would persist in meat and also known to transferred to milk (Szabo and Tebbett 2002). Human beings might potentially exposed to L-Dopa through these milk or meat, thereby there may be chance to transmission of L-Dopa from livestock to human beings, but till date no studies have addressed this concern. Furthermore, concern for the well-being of livestock suddenly exposed to high levels of L-Dopa via feed or

fodder appears to be justified.

Matenga *et al.* (2003) conducted a lactation trial with indigenous Mashora does (goats) with the body weight of 23-45 kg. The study reveal that there is no difference between the goat kids fed with milk secreted from goats receiving *Mucuna* diet and control diet. This indicates that either L-Dopa was not present in the milk, or it was present in very meager quantities. Further evidence of a decrease in L-Dopa might be concluded by the similar lack of effect on doe milk yield by *Mucuna* diets, even after six weeks. Some L-Dopa was absorbed by the does, however, as evidenced by the slight darkening of their urine, assumed to be due to melanin excretion. Since, the L-Dopa is a precursor of melanin, during metabolic reactions it may converted into melanin pigments (Spratto and Woods 1996; Pfutzner and Przybilla 1997; Letellier *et al.* 1999; Hachinohe and Matsumoto 2007). Zhao *et al.* (2007) reported that the L-Dopa generates quinines that polymerize to form melanin. Both the L-Dopa and L-Tyrosine are precursors for the biological pigment melanin. The enzyme tyrosinase catalyzes the oxidation of L-Dopa to the reactive intermediate dopaquinone, which reacts further and eventually leading to the formation of melanin pigments.

As a feed, L-Dopa does not appear to be a problem for ruminants. While ruminants seem to consume feed containing high levels of L-Dopa without problems, the mechanisms that enable this consumption was not understood. Since the fate of L-Dopa in the ruminant track is not understood, the information is not available on whether it is passed through milk. However, due to its rapid elimination from the body it seems unlikely that it would persist in the meat of animals (Burgos *et al.* 2002). Only a minor deleterious effect of L-Dopa on the performance of cattle is confirmed by the results of Burgos *et al.* (2002). Most studies conducted to date have been of a short-term, are poorly understood and incomplete. L-Dopa should be avoided during pregnancy and by nursing mothers, since the compound can appear in the milk (Standaert and Young 1996). Kageyama *et al.* (2000) reported that the L-Dopa was transported across the blood-brain barrier by amino acid transporter system.

The negative effects of L-Dopa can be alleviated through suitable processing of the seed and by giving a period of acclimation (gradual increase in *Mucuna* feeds) similar to that required by humans taking L-Dopa as a treatment regimen. Prior to the use of LAAD inhibitors, the maximum human exposure during supervised treatment was 8 g of L-Dopa/day for a average body weight of 70 kg man (0.1 g/kg/day). This level was known to have fairly severe side effects. Current maximum dosage levels with the inhibitors have decreased to 2-3 g/day (0.03-0.04 g/kg/day). Animal feeding studies showed that the calculable average daily ratios ranged from ~0.4 for pigs (Flores *et al.* 2002) and over 30 g L-Dopa/kg body weight for broilers (del Carmen *et al.* 1999). However, details regarding possible exposure levels of cows to L-Dopa has not yet been studied.

To study the biological effect of L-Dopa, a rat feeding experiment was conducted with a total of 168 weanling, 22 day-old white rats, with an average initial weight of 44 g (Bressani *et al.* 2003). On the basis of diet consumed, on the amount of *Mucuna* flour incorporated in the diet, and the L-Dopa content of the bean flour, L-Dopa intake was

calculated. This was then expressed as mg of L-Dopa ingested daily and also expressed on a per kg body weight of the rats. In the first trial, the daily L-Dopa average intake was 121 mg (range 75-166 mg) or 2450 mg/kg body weight (2083-3667 mg/kg); in the second trial with roasted *Mucuna* bean flour, daily L-Dopa intake averaged 74 mg (69-82 mg) and total average intake was 1941 mg/kg (range 1605-2242 mg/kg). In the third study with germinated beans daily L-Dopa intake averaged 119 mg (88-161 mg) or 3126 mg/kg (2667-3833 mg/kg). No relationship between weight and L-Dopa intake was suggested by the data and all values of intake fell below the 50% lethal dose of 4000 mg/kg body weight was determined according to the method described by Budavaris *et al*. (1989). The daily intakes were also below initial doses given to humans with Parkinson's disease of 250 mg/day (Moffat 1986; Spratto and Woods 1996; Dollery 1999).

Rat mortality was associated with a high trypsin inhibitor activity rather than with L-Dopa content, which was not affected by the various processing methods applied. On the other hand, weight change differences for the other treatments were probably more related to changes in amino acid availability, mainly lysine, which is known to be reduced by excessive moist or dry heat processing. Daily administration of L-Dopa to mice beyong 2-3 weeks of age was reported to stimulated mounting and aggressive behaviour of adult males towards both male and female mice (Szczypka *et al*. 1998).

People and monogastric animals consuming improperly processed *Mucuna* seeds, are known to suffer from gastrointestinal disturbances, notably, nausea, vomiting, and anorexia, as well as more serious effects, such as paranoid delusions, hallucinations, delirium, and unmasking of dementia (Reynold 1989). The long-term impact of L-Dopa on human health is not known, in particular when L-Dopa is ingested in foods consumed by a cross section of a mainly healthy population, including both young and old, but also persons with various illnesses. Some of the results generated by studies on the treatment of Parkinson's disease with L-Dopa indicated that indeed, extremely low levels of L-Dopa must be the goal of any food-oriented research. The review by Szabo and Tebbett (2002) points out those individuals with asthma, psychosis, peptic ulcers, epilepsy, cardiovascular and pulmonary diseases, and diabetes should avoid L-Dopa. It may also cross the placental and blood-to-milk barriers, thus impacting the health of infants. Since most of the medical research has been conducted on those with Parkinson's disease, there is little information on the impact of L-Dopa on healthy individuals to determine a safe maximum daily dose of L-Dopa when ingested in foods by large segments of the population. The side effects of L-Dopa in humans have been associated with dizziness, staggering, increased heart rate, vomiting, and psychiatric disturbances, which was consistent with it being on the synthetic pathway of the neurotransmitter dopamine (Standaert and Young 1996; Dollery 1999; Metman and Mouradian 1999; Szabo and Tebbett 2002).

Elwan and Sakuradawa (2007) tested the ability of monkey's amniotic epithelial cells to take up and decarboxylate the L-Dopa by incubating the cells in buffer containing L-Dopa under different experimental conditions followed by assaying the cellular dopamine content using HPLC with electrochemical detector. Cellular contents of dopamine were significantly increased in a time- and L-Dopa concentration-dependent manner, suggesting the uptake of L-Dopa by monkey's amniotic epithelial cells.

In mammalian cells the L-Dopa has been reported to cause cell death by generating reactive oxygen species during its oxidative polymerization into melanin. The phototoxicity of L-Dopa might be due to reactive oxygen species generated by melanin synthesis pathway (Hachinohe and Matsumoto 2007). Pavlis *et al*. (2006) showed that intraperitoneal injections of L-Dopa (20-50 mg/kg) promote rats odor discrimination performance in comparision to control rats injected with saline. The L-Dopa was reported to

transferred across the blood-brain barrier by an amino acid transporter system (Kageyama *et al*. 2000). The intestinal absorption of L-Dopa in human beings was evaluated by using a newly developed *in vivo* intestinal perfusion instrument by Lennernas *et al*. (1992).

A clearer picture about the safe level of L-Dopa in the diet is beginning to develop from research on acute symptoms of L-Dopa toxicity (e.g., head aches, nausea after a meal or inferior performance in animal feeding trials). Even though a lot of medical research has been done on L-Dopa, it has been with people who have a neurological disease. It is worth noting that we may already have two millennia or more of experience with people consuming low quantities of L-Dopa. This is also the case with pregnant women, with regard to potential fetal effects. Even though it is believed that L-Dopa can cross the placenta (Cook and Klawans 1985; Lennernas *et al*. 1992; Kageyama *et al*. 2000), there is almost no data on humans. For the remaining healthy population the safe level would seem to vary. Overall nutritional health seems to be a factor along with the types of foods that are ingested during L-Dopa exposure.

As it is evident from the review of Szabo and Tebbett (2002), although there is medical literature available on the impacts of L-Dopa on human health, available medical data was almost exclusively derived from short-term trials with individuals who have Parkinson's disease. No work had been conducted on the impacts of L-Dopa on a cross-section of population consuming small amounts of L-Dopa over long periods of time. Typically among the Parkinson treated patients there was a period of adjustment to L-Dopa medication during which even small doses (0.1 g L-Dopa/Kg/day) of the medicine produced strong side effects. Whether non-ruminant animals may undergo a similar adjustment period, and what the impact of higher tolerance to L-Dopa content in the feeds might be, remains to be studied.

The amount of L-Dopa that would be ingested by drinking a cup of *Nutricafe*, a coffee substitute prepared by toasting the *Mucuna pruriens* seed flour with cinnamon and brown sugar, available in Central America was estimated (Myhrman 2002). It was predicted that an individual consuming 250 ml of *Nutricafe* would ingest more than 250 mg of L-Dopa. The remaining data suggested that this assumption is reasonable, since the amount of L-Dopa extracted was neither decreased significantly by using room-temperature water, nor increased by boiling for 12 min after the initial addition of boiling water. To provide a context for these numbers, Szabo and Tebbett (2002) reported that 250 mg/day is a typical starting dose of L-Dopa for the treatment of Parkinsonism.

Currently, products containing *Mucuna* are being sold for a number of purposes through the Internet, including increased muscle mass and strength, male vitality, enhancement of mental alertness and coordination, as well as for an alleged anti-aging effect. But no reliable evidences are available to confirm these benefits of L-Dopa. On the other hand the antioxidant property of L-Dopa was proved by Siddhuraju and Becker (2003b). The polyphenolic constituents of various legume seeds have been reported to have potential medicinal properties, including antioxidant activities. The antioxidant potential and effective hydroxyl radical scavenging ability of phytochemical extracts from various common legumes such as *Phaseolus vulgaris* (Tsuda *et al*. 1994); mung bean (Duh *et al*. 1997); pea nut (Yen and Duh 1993); horse gram (Siddhuraju and Manian 2007); *Vigna unguiculata* (Siddhuraju and Becker 2007); *V. aconitifolia* (Siddhuraju 2006) and other edible pulses (Lee *et al*. 2000) and various under-utilized legume seeds such as *Lathyrus sativus* (Gongke *et al*. 2001), *Mucuna pruriens* (Tripathi and Upadhyay 2001; Siddhuraju and Becker 2003b) and *Tamarindus indica* (Siddhuraju 2007) were already reported. Effective hydroxyl scavenging ability of the non-protein amino acid β-N-Oxalyl-L-α,β-diaminopropionic acid (β-ODPA) in *Lathyrus sativus* was reported by Gongke *et al*. (2001).

When considering the scavenging effect on DPPH (α,α-

diphenylamine-β-picrylhydrazyl) radicals, L-Dopa was found to be a more potent DPPH scavenging than all other compounds of *M. pruriens* tested including the reference standards. Siddhuraju and Becker (2003b) also studied the non-enzymatic generation of superoxide anion radical scavenging activity of L-Dopa by Nitrobluetetrazolium (NBT) reduction assay. Superoxide is a biologically important substance, which can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Korycka-Dahl and Richardson 1978). The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins (Spencer *et al*. 1994). The results of Siddhuraju and Becker (2003b) study reveal that the L-Dopa inhibit superoxide radicals in a dose-dependant manner.

Spencer *et al*. (1996) reported the pro-oxidant and antioxidant actions of L-Dopa and its metabolites such as dopamine, melanine, norepinephrine, 3-methoxytyramine, 3-*O*-methyldopa, 3,4-dihydroxyphenyl acetic acid and 3-methoxy-4-hydroxy phenyl acetic acid promoted oxidative DNA damage and could also be harmful to tissues damaged by neurodegenerative disease, namely Parkinsonism. Moreover, a study through *in vitro* models revealed that L-Dopa significantly increases the levels of oxidized glutathione in rat brain striatal synaptosomes (Spina and Cohen 1988). Such depleted glutathione could be due to the generation of reactive semiquinones from L-Dopa. The reducing power of L-Dopa might be due to their di- and mono-hydryl substitutions in the aromatic ring, which possess potential hydrogen-donating abilities. Thus, this non-protein amino acid might contain higher amounts of reducing reductones and could react with free radicals to stabilize and terminate radical chain reactions.

Spencer *et al*. (1994) reported that L-Dopa, which is rich in the nervous system, and its metabolites dopamine and 3-*O*-methyL-Dopa cause extensive oxidative DNA damage in the presence of H_2O_2 and traces of copper ions via promotion of lipid peroxidation and highly reactive hydroxyl radicals. Nonetheless, chronic treatment with L-Dopa was reported to decrease lipid peroxidation in the cerebral cortex of healthy mice, but to worsen peroxidation in mice with neuronal injury and thus L-Dopa appears to have a mixture of pro- and antioxidant effects *in vivo*, which means that it could be harmful to tissues damaged by neurodegenerative diseases.

Oxidative stress plays an important role in the pathogenesis of Parkinson's disease. The intake of L-Dopa was negatively dose related to endogenous and exogenous plasma lipid peroxidation. The plasma of Parkinson's patients has elevated levels of lipid peroxidation and is more to peroxidation than that of control group, which suggest the antioxidant effect of L-Dopa (Agil *et al*. 2006). Using the single-cell gel electrophoresis ("Comet") assay, Shi *et al*. (2002) showed that tyrosinase-generated L-Dopa oxidation products prevent H_2O_2-induced oxidative DNA damage in cultured tissue cells. We propose that these oxidation products trigger cellular processes that up-regulate the overall antioxidant status of the cell, and could be incorporated into treatments of pathological conditions associated with elevated oxidative DNA damage and other manifestations of increased oxidative stress.

The autooxidation of L-Dopa generally yields reactive oxygen species and neurotoxic quinines. NADPH-Quinone-Oxidoreductase (NQO) is a flavoenzyme that is implicated in the detoxification of quinines. Through the action of this enzyme, deleterious redox-labile quinines are turned into less toxic and more stable hydroquinones that are amenable to further detoxification and cellular excretion. Muiswinkel *et al*. (2000) reported that the L-Dopa stimulates the expression of antioxidant enzyme NQO. Pardo *et al*. (1995) reported that the antioxidants such as ascorbic acid and sodium metasulfite completely prevented the L-Dopa induced quinine formation as well as death of non-dopamine neurons.

One practical approach in addressing the issue of safe target levels is to simply adopt the levels of L-Dopa found in the common fava bean or broad bean. Although cooked fava beans seem to alleviate symptoms of Parkinson's disease (Rabey *et al*. 1993), there are no known long-term negative effects from its consumption. Therefore, fava bean foods could serve as a guide in determining what maximum level of L-Dopa needs to be the objective.

A study on the accumulation of vicine, convicine and L-Dopa in the pod, cotyledons and seed coat during pod development of *V. faba* showed that the L-Dopa content was very high in young pods of cvs. 'Alameda' and 'Diana' and its concentration decreased rapidly during the drying phase to reach a level of 0.5% when the dry matter was close to 80%. The concentration of L-Dopa showed a significant negative correlation with dry matter percentage in both cultivars (Burbano *et al*. 1995). The loss of L-Dopa during the drying of plant sample was observed by many workers (Longo *et al*. 1974; Larher *et al*. 1984). Because of the high content of L-Dopa in the green pods of *V. faba*, it was also used in the treatment of Parkinson's disease (Larher *et al*. 1984). However, it is necessary to be careful with the rate of consumption because L-Dopa plays a certain role in favism disease development (Mager *et al*. 1980).

V. faba beans also contain L-Dopa, but to a lesser extent than *Mucuna* beans, i.e., in the order of 0.2-0.5% dw basis. Cooked faba beans and processed foods have allowed them to be safely consumed by a large number of people in many parts of the world over generations. On this basis, a concentration of L-Dopa at 0.1% dry solids was adopted as a level which processing should reach. This means that any effective extraction process must be capable of achieving approximately 99% reduction in initial L-Dopa levels. The previous reports show that the effectiveness of L-Dopa from faba pods (which contain more L-Dopa than faba beans) or beans on the people with Parkinson's disease with out any medical problems (Rabey *et al*. 1993). It may become apparent that a large population in the middle east is consuming L-Dopa in faba pods regularly and without problems, and it may be possible to determine at what frequency and amounts the L-Dopa is being safely consumed (Szabo and Tebbet 2002). Clearly, very low levels of L-Dopa do not negatively impact health, as evidenced by the long use of *V. faba* (which also contains L-Dopa, albeit at a much lower concentration) by large populations.

The reports of Gilbert (2002) and Price (2002) suggested that the maximum safe level of L-Dopa for a healthy adult consume per day is 1.5 g/person/day. But for a number of reasons 1.5 g/person/day seems too high and, as pointed out by Szabo and Tebbett (2002), is based on data from short-term studies. Long-term impacts on healthy humans at similar L-Dopa levels are unclear. Moreover, more severe side effects are tolerated when a compound is used to treat symptoms of a serious disease than when such compound is to be part of normal healthy diets. In addition, a general population consuming products containing L-Dopa would always include individuals with various ailments who should not, according to current medical knowledge, be exposed to L-Dopa. Finally, little is known about the impacts of L-Dopa on fetuses or breast feeding babies. The daily intakes were also below initial doses given to humans with Parkinson's disease of 250 mg/day. Lorenzetti *et al*. (1998) also reported that maximum tolerable limit for L-Dopa of 1.5 g/person/day. Thus 100 g of unprocessed *Mucuna* seed would contain intolerable amounts of L-Dopa. For L-Dopa, researchers have suggested levels below 1% (Versteeg *et al*. 1998) or that the daily consumption should not exceed 1500 mg per person (Lorenzetti *et al*. 1998). However, no long term studies have been conducted to determine safe consumption levels of *Mucuna* foods.

DETOXIFICATION METHODS

Since the presence of L-Dopa is widely regarded as the major obstacle in causing various side effects and toxic properties in nutritional point of view, various processing methods that significantly reduce its concentration to a thrust hold level (a safe level of L-Dopa which does not exhibit

Table 4 Effect of various processing methods on the L-Dopa content of velvet beans.

Name of the Processing method	% of L-Dopa loss
Soaking in tamarind extract solution[1]	8-17
Soaking in citric acid solution[1]	5-7
Soaking alkaline solution[1]	15-16
Soaking in distilled water[1]	6-8
Soaking in water + Dehusking[1]	2-3
Autoclaving[1,2]	15-59
Soaking in water + Cooking[1]	56-63
Soaking in water + Autoclaving[1]	60-63
Soaking in tamarind extract solution + Autoclaving[1]	56-61
Soaking in tamarind extract solution + Cooking[1]	41-56
Soaking in alkaline solution + Autoclaving[1]	65-61
Soaking in alkaline solution + Cooking[1]	60-62
Soaking in Citric acid solution + Autoclaving[1]	60-70
Soaking in Citric acid solution + Cooking[1]	45-48
Dry heating[1]	10-26
Germination for 24 h[1]	13-16
Germination for 48 h[1]	17-19
Germination for 72 h[1]	20-23
Sequentially Cooking[1]	56-60
Soaking in NaHCO$_3$ solution[2]	17-20
Soaking in NaCl solution[2]	14-1528
Cracked seeds soaked for 1 day + Dehusked[3]	69
Soaked in 4 % Ca(OH)$_2$ solution for 24 h[3]	99
Cracked seeds soaked in 4 % Ca(OH)$_2$ solution for 24 h[3]	49
Roasted for 15 min.[4]	47
Roasted for 20 min.[3]	31
Toasted for 30 min. at 130ºC[5]	12
Boiled for 45 min. + Dehulled[6]	26
Boiled for 45 min. + Soaked for 12 h[7]	64
Boiled for 45 min. + Soaked for 12 h[7]	78
Soaked for 45 min. + Boiled for 45 min.[7]	90
Boiled in Magadi solution[8]	59
Boiled in Citric acid solution[8]	49
Boiled with maize cob ash[8]	58
Boiled in bean stover ash[8]	47
Germination for 5 days + Dehulling + Boiling[8]	37
Germination for 7 days + Dehulling + Boiling[8]	41
Whole seeds soaked in faucet water for 72 h[9]	95
Cracked seeds soaked in faucet water for 72 h[9]	99
Cracked seeds soaked in river water for 72 h[9]	94

[1]Siddhuraju and Becker (2001); [2]Janardhanan *et al.* (2003); [3]Diallo *et al.* (2002); [4]Gilbert (2002); [5]Flores *et al.* (2002); [6]Del Carmen *et al.* (1999); [7]Egoulenty (2002); [8]Wanjekechi *et al.* (2003); [9]Diallo and Berhe (2003).

any deleterious effect on the consumers) would seem to provide a possible approach to this problem. To date, although various processing techniques were designed for the removal of L-Dopa (**Table 4**), the scientific knowledge and understanding of basic principles of involved in the removal/reduction of L-Dopa during the processing method has not been explored. Hence, this part of review focused on the science perspective what is already known about destruction and/or extraction of L-Dopa from *Mucuna* beans and discusses the scientific principles underlying these process technologies, currently available technology in commercial use for such processing operations was described and the application of these basic principles to potential process treatment concepts based on utilizing appropriate technology was also discussed. To date, a number of methods have been employed, however, as pointed out by Balaban and Teixeira (2002), processing to decrease L-Dopa has mainly relied on methods of trial and error.

Soaking in water and various solutions

Janardhanan *et al.* (2003b) studied the effect of soaking the seeds samples in NaHCO$_3$ solution on the L-Dopa content of five different accessions of velvet bean seeds collected from South India and they found that the reduction of 17-

20% reduction in the L-Dopa content of velvet bean seeds. Soaking in 0.1% NaCl resulted in the reduction of 14-15% of L-Dopa content in velvet bean seeds (Janardhanan *et al.* 2003b). Soaking the velvet bean seeds in NaHCO$_3$ for 12 h and 24 h reduced the L-Dopa content from 4.1% to 4.1% and 3.8%, respectively (Nyirenda *et al.* 2003). Soaking the velvet bean seeds in water for 20 h at 25ºC was reported to reduced the L-Dopa content of 8.7% and 6.1% in white and black accessions of velvet beans, respectively (Siddhuraju and Becker 2001a). Similarly, Vijayakumari *et al.* (1996) reported the reduction of 16% and 18% of L-Dopa when the velvet bean seeds were soaked for 9 h in distilled water and NaHCO$_3$ solution, respectively.

Diallo and Berhe (2003) found the impact of soaking time and running water from faucet or in a river on the L-Dopa content of whole and cracked velvet bean seeds. They reported that soaking the whole seeds in faucet water for 72 h reduced the L-Dopa content from 4.93 % to 0.23 %. The L-Dopa content of cracked seeds (4.3%) was reduced to 0.04% when the seeds were soaked in faucet water while soaking in river water for 72 h reduced the L-Dopa to 0.26%. Soaking the velvet bean seeds in water for 12 h and 24 h reduced the L-Dopa level from 4.1% to 4.0% and 3.8%, respectively (Nyirenda *et al.* 2003). Soaking the velvet bean seeds for 6h in distilled water was found to reduced 19% of L-Dopa content (Vadivel and Pugalenthi 2007).

The L-Dopa content in white and black seeds of velvet bean was reduced to 8.7% and 17.3%, 14.9% and 15.9%, 6.8% and 5.1% when the samples are soaked in tamarind pulp extract, NaHCO$_3$ solution and citric acid solution, respectively (Siddhuraju and Becker 2001a). Soaking in tamarind pulp extract and NaHCO$_3$ solution improved the reduction of L-Dopa level significantly when compared to citric acid solution and water soaking treatments. Gurumoorthi *et al.* (2007) reported that soaking the velvet bean seeds in Ca(OH)$_2$ solution for 6 h reduced the L-Dopa content from 6 to 54%. Srivastava and Khokhar (1996) have also observed that the significant effect of tamarind pulp extract and NaHCO$_3$ solution soaking on the reduction of an another non-protein amino acid, β-ODAP in *Lathyrus sativus*. D'Mello and Walker (1991) also reported that the seeds of *Canavalia ensiformis* when subjected to soaking in potassium-bi-carbonate solution at 80°C, the concentration of an another non-protein amino acid, Canavanine was declined to negligible level within 48 h.

Such a low and variable reduction of L-Dopa in the velvet bean seeds during soaking in water and various solutions could be explained by two factors: 1. the permeability of the seed coat along with the diffusion rate of L-Dopa in different ionic strength solutions and 2. The presence of L-Dopa in the intact cell compartments of the cotyledons rather than the seed coat. The second factor might be more pronounced on the least leaching of L-Dopa during various soaking treatments, although it is a water soluble compound (Siddhuraju and Becker 2001a).

Hydrothermal treatments

Cooking the velvet bean seeds for 2 h, 4 h and 6 h resulted in the reduction of L-Dopa content from 5.61% to 5.6%, 3.7% and 2.7%, respectively (Bressani *et al.* 2003). Cooking the velvet bean seeds for 90 min was reported to reduce 20% of L-Dopa content and 45 min of cooking reduced 15% of L-Dopa content (Vijayakumari *et al.* 1996). Sequential cooking reduced 60% and 56% of L-Dopa in white and black velvet bean seeds, respectively (Siddhuraju and Becker 2001a).

Autoclaving the velvet bean seeds for 30 min at 15 lb pressure was found to reduce 25% of L-Dopa content (Siddhuraju *et al.* 1996). 15% of reduction of L-Dopa was reported during autoclaving treatment by Janardhanan *et al.* (2003b). Autoclaving was found to reduce the L-Dopa content of 51% in white seeds and 59% in black seeds of velvet beans (Siddhuraju and Becker 2001a). Autoclaving for 15, 30 and 45 min reduced the L-Dopa content of 9%, 15% and

22% in *M. pruriens* seeds (Vijayakumari *et al.* 1996). Reduction of 79% of L-Dopa content was recorded during the velvet bean seeds autoclaved at 15 lb pressure (121°C) for 30 min (Vadivel and Pugalenthi 2007).

Cooking the water soaked velvet bean seeds for 2 h, 4 h and 6 h reduced the L-Dopa content from 5.08% to 4.89%, 3.7% and 2.2%, respectively (Bressani *et al.* 2003). Soaking the velvet bean seeds for 48 h in water and cooking for 3 h and 6 h reduced the L-Dopa level from 5.1% to 3.9% and 4.9%, respectively. Soaking the velvet bean seeds for 48 h in water and cooked for 30 min, 45 min and 60 min at 15 lb pressure increases the L-Dopa from 5.15% to 5.8%, 5.7% and 5.5%, respectively (Bressani *et al.* 2003). Cooking the velvet bean seeds for 1h in the bean to water ratio of 1:10 (w/v) resulted in the 39% reduction of L-Dopa (Vadivel and Pugalenthi 2007).

Soaking the velvet bean seeds in water followed by boiling for 30 min reduced the L-Dopa content from 4.1% to 2.1%. The L-Dopa content was reduced from 4.1% to 1.9% during soaking the velvet bean seeds in NaHCO$_3$ solution followed by boiling for 30 min. Soaking the seeds of velvet beans in water for 1 h, boiling it for 30 min followed by soaking in water for 30 min reduced the L-Dopa content from 4.1% to 2.0%. Soaking the seeds of velvet beans in NaHCO$_3$ for 1 h, boiling it for 30 min followed by soaking in NaHCO$_3$ for 30 min reduced the L-Dopa content from 4.1% to 1.3% (Nyirenda *et al.* 2003).

Reduction of significant levels of L-Dopa was observed during various treatments such as soaking in water followed by autoclaving (60% and 63%), soaking in water followed by cooking (56% and 63%), soaking in tamarind pulp extract followed by autoclaving (56% and 61%), soaking in tamarind pulp extract followed by cooking (41% and 56%), soaking in NaHCO$_3$ solution followed by autoclaving (65% and 61%), soaking in NaHCO$_3$ solution followed by cooking (60% and 62%), soaking in citric acid solution followed by autoclaving (60% and 70%) and soaking in citric acid solution followed by cooking (45% and 49%) in both white and black seeds of velvet beans, respectively (Siddhuraju and Becker 2001a).

Siddhuraju and Becker (2005) was reported that the reduction of 72%, 74%, 68% and 70% of L-Dopa during soaking in water, 0.07% NaHCO$_3$, 0.1% ascorbic acid solution and water containing 3% freez-dried moringa leaf powder followed by autoclaving. Janardhanan *et al.* (2003b) reported that 56-60% of L-Dopa was reduced in the velvet bean seeds during repeated boiling. Boiling the velvet bean seeds for 30 min reduced the L-Dopa content from 4.1% to 2.1% (Nyirenda *et al.* 2003).

Ukachukwu and Szabo (2003) studied the effect of addition of various additives such as Magadi salt and wood ash during boiling on the L-Dopa content of the velvet bean seeds. They reported that boiling with additives such as Ca(OH)$_2$, Magadi (a hydrated sodiumbicarbonate collected from Lake Magadi, Kenya) and wood ash for 90 min reduced 64.2%, 64.6% and 66% of L-Dopa content of velvet beans, respectively. Wanjekeche *et al.* (2003) reported that the boiling the whole and sliced velvet bean seeds in Magadi solution (Magadi soda is a hydrated sodium carbonate obtained from the Lake Magadi, Kenya) for 30 min results in the 59% and 80% reduction of L-Dopa, respectively. Boiling in citric acid solution for 1 h resulted in 49% removal of L-Dopa in the whole seeds and 69% of removal of L-Dopa in sliced velvet bean seeds. Boiling in maize cob ash reduced the L-Dopa level of 58% in whole beans and 74% in sliced velvet beans. Boiling the beans in stover ash reduced 47% of L-Dopa in whole beans and 69% of sliced velvet bean seeds.

Dry heating

Roasting *M. pruriens* seeds for 15 min removed 49% of L-Dopa (Gilbert 2002) while roasting for 20 min resulted in the removal of 35-47% of L-Dopa (Diallo *et al.* 2002). Roasting the velvet bean seeds for 10, 20 and 30 min re-

duced the L-Dopa content from 5.3% to 3.79%, 3.74% and 4.64%, respectively (Bressani *et al.* 2003). Roasting of velvet bean seeds was reported to reduce 10% and 26% of L-Dopa in white and black seeds, respectively (Siddhuraju and Becker 2001a). Siddhuraju *et al.* (1996) reported that dry heating reduced 45% of L-Dopa from the velvet bean seeds. Toasting the velvet bean seeds for 30 min at 130°C was reported to reduced 12-31% of L-Dopa (Flores *et al.* 2002; del Carmen *et al.* 1999). 10 to 34% of reduction in the level of L-Dopa content of velvet bean during dry heat treatment was observed by Gurumoorthi *et al.* (2007). Roasting the velvet bean seeds in an iron pot at 100°C was found to reduced 50% of L-Dopa in the velvet bean seeds (Vadivel and Pugalenthi 2007).

Germination

Germination of velvet bean seeds for 2 h, 4 h and 6 h resulted in the reduction of L-Dopa content from 5.1% to 4.5%, 4.4% and 4.3%, respectively (Bressani *et al.* 2003). Germination of presoaked seeds for 24 h, 48 h and 72 h resulted in reduction of L-Dopa content of 13-16%, 17-19% and 20-23%, respectively (Siddhuraju and Becker 2001a). Prakash and Tewari (1999) who reported percentage L-Dopa loss of 17%, 31%, 45% and 72% at 1, 3, 5 and 7 days of germination of *M. pruriens* seeds. The changes in L-Dopa content upon germination are shown that there was a small decrease in L-Dopa content, from 5.11% to 4.37%, but it was not significant (Diallo *et al.* 2002). These results suggested that enzymes are not developed upon germination to metabolize L-Dopa as in the case for phytase on phytic acid (Eskin and Wiebe 1983).

The L-Dopa content of the malted samples was no different than that of samples that had been only germinated. Germination for 5 days combined with boiling reduced the L-Dopa by only 37%. Increasing the duration of germination to 7 days further reduced the L-Dopa content by 41.7%. Lastly, germination and malting the beans did not significantly reduce L-Dopa levels, and resulted in beans that were similar to the raw beans in terms of nutritional value and biological value (Prakash and Tewari 1999). A 34-58% of reduction of L-Dopa during germination of velvet bean for 120h was reported by Gurumoorthi *et al.* (2007).

Enzymatic breakdown of L-Dopa

Considerable evidence exists to support the efficacy of enzymatic degradation of L-Dopa. The activity of polyphenol oxidase (PPO) enzyme on the L-Dopa compound was studied by Chen *et al.* (1991). Basically this enzyme acts on the L-Dopa and reducing it to some other less toxic substances. Other specific enzyme studied by Chen *et al.* (1991) was tyrosinase, which also reported as potential tool to degrade the L-Dopa of velvet bean seeds.

Fermentation

Effect of fermentation on the L-Dopa content of velvet bean was studied by Egounlety (2003). The level of L-Dopa was increased significantly during the first 12 h of *Mucuna tempe* fermentation and the first 24 h of *Mucuna condiment* fermentation. It almost doubled during the above-stated fermentation periods, varying from 0.639% to 1.240% and from 0.776% to 1.197%, respectively. It thereafter decreased more significantly in the *tempe* than in the condiment fermentation. Fermentation of maize-velvet bean (and melon) with natural lactic acid bacteria had no effect on L-Dopa. The increase in L-Dopa during the early stages of *Mucuna tempe* or condiment fermentations might be due to the release of bound L-Dopa.

A similar observation was made by Roozen and de Groot (1985) on the trypsin inhibitor activities (TIA) during soybean *tempe* fermentation. They reported an increase of 0.17% and 0.23% of trypsin inhibitor activities after 24 and 48 h incubation, respectively. In fungal fermentation, the

significant reduction observed thereafter suggested the production of an L-Dopa degrading enzyme (Egounlety 2003). In *Mucuna* condiment fermentation, the rapid change in pH towards the alkaline environment might explain the decrease in L-Dopa and the deep darker colour of the 48 h fermented condiment. Siddhuraju and Becker (2001a) reported that L-Dopa and other *Mucuna* compounds are readily oxidizable at alkaline pH, high temperature (70-100°C) with more moist conditions and form dark-coloured compounds. Most of the dark colour, assuming there has been no charring, is likely from melanin, breakdown products of L-Dopa and other closely structured indolic alkaloids (Egounletey 2003).

In Japan the use of *Mucuna* in *tempe* production has been reported (Higasa *et al*. 1996). 70% of L-Dopa from the velvet bean seeds was reduced in *tempe* fermentation. Egounlety (2003) proved that ensilage fermentation decreases L-Dopa content by 10-47%. There is evidence for the involvement of lactic acid bacteria in markedly decreasing the L-Dopa content during the fermentation of *Mucuna* in the production of weaning food. There was an apparent diminishing of this reducing effect on L-Dopa with decreesing maize content. Fermentation was probably less intense with lower carbohydrate content, and this in turn may have less effect on L-Dopa. It would seem that fermentation with good levels of water soluble carbohydrates was required to have the best effect on L-Dopa. Randhir and Shetty (2007) reported that the L-Dopa content of mung beans was increased (1.2 mg/g) from the initial content (0.6-0.7 mg/g) during solid state fermentation with *Rhizopus oligosporus* and also they observed that the antioxidant activity of L-Dopa was also increased up to 90% on the 12th day of fermentation.

Solvent extraction

Solvent extraction is widely used in the food, pharmaceutical and chemical industries to extract a soluble constituent from a solid substance by means of intimate contact with a liquid solvent. Such processes are often referred as leaching, steeping, brewing or diffusion such as in the brewing of coffee, steeping of tea, diffusion of sugar from sugar beet and the solvent extraction of vegetable oil from oil seeds and grains or vanilla extraction from the vanilla beans. The following factors are important in explaining the efficiency of extraction processes, and could therefore be manipulated in the case of L-Dopa extraction.

Particle size of the solid material significantly influences the rate of extraction in a number of ways. The smaller the size, the greater the interfacial surface area at the solid-liquid interface and therefore the greater the rate of transfer of solubles at the surface. The smaller size also reduces the distance to be traveled by the solute molecules within the particle interior as they migrate toward the surface (diffusion). However, when taken to extremes, such as with very fine powders, the circulation of liquid solvent around the particles can be impeded, thus compromising the benefit of greater surface area (Balaban and Teixeira 2002).

Agitation of the solvent is important because it increases the eddy diffusion (turbulence) and relative velocity of liquid at the particle surface, thus increasing the rate of transfer of solute from the particle surface to the bulk solution. At the same time, this rapid removal of solute from the surface maintains the maximum concentration gradient needed between surface and interior to maximize the internal rate of solute diffusion from the particle interior to the surface.

Viscosity of the liquid chosen as solvent should be relatively low in order to circulate freely. Concentration of solute in solvent should be as low as possible. Generally, a relatively pure solvent is used initially, but as extraction proceeds, the concentration of solute in the solvent will increase thus reducing the concentration gradient (driving force), and the rate of extraction will progressively decrease in an exponential decay. Most large-scale industrial pro-

cesses minimize this problem by using a continuous flow of fresh pure solvent in a multistage countercurrent continuous system. Temperature can affect the rate of extraction. In many cases the solubility of the material being extracted into the solvent will increase with temperature to give a higher extraction rate. For this reason, heat is often added to maintain elevated temperatures.

In the case of extracting L-Dopa from velvet beans, the primary product of value will be the spent residue while the liquid solvent rich with dissolved L-Dopa is the disposable waste or by-product. A first step in attempting to apply the basic principles discussed earlier to the solvent extraction of L-Dopa from *Mucuna* beans is to identify potential solvents for L-Dopa, and determine the solubility of L-Dopa in the selected solvent(s). Given the resource limitations of the smallholders in need of this processing technology, it is clear that water at ambient temperatures would be the liquid solvent of choice. Other possibilities that may be economically feasible would be mildly acidic or alkaline solutions. The solubility of L-Dopa in these solvent systems is discussed in the following sections.

L-Dopa extraction with water

Teixeira *et al*. (2003) studied the optimum conditions for extracting the L-Dopa from velvet beans and the effect of bean particle size, water circulation rate, water temperature and pH on the extraction rate of L-Dopa was also determined. Information on the solubility of L-Dopa was found in the Merck Index (1983), which indicated it has only limited solubility in water (66 mg in 40 ml). Assuming an initial content of L-Dopa in *Mucuna* bean in the range of 3-6% dry weight, this translates into the need for 20 to 40 parts of water to one part seed by weight (20-40 liters of water for each kg of beans) in a batch extraction process in order not to reach the solubility limit in water. This fact alone may explain part of the limited success in water extraction efforts at the village level. None the less, if sufficient water is used along with small particle size and agitation, extraction rates can be quite high. For example, Laurent *et al*. (2002) reported complete extraction of L-Dopa from *Mucuna* in less than 5 minutes by placing 0.1 g of powdered seed into 15 mL of distilled water (150 parts water to 1 part seed) and placing it in an ultrasonication bath for 5 minutes, but however, it is not economical and not feasible for small-holder farmers.

Effect of particle size

Even after 24 h of water extraction, the larger bean particles show little more than 50% reduction, which occurs within the first 2 h and tends to level off. Only the very smallest 1 mm particle size samples show a decline reaching a level of 0.25% (90% reduction) in 24 h. Results of this study exhibit a relatively rapid rate of decline early in the extraction process that continually diminishes with time indicates that the extraction may be following a pattern of exponential decay. This is to be expected because as extraction proceeds, the concentration of solute (L-Dopa) in the solvent (water) will increase, thus reducing the concentration gradient (driving force) causing the rate of extraction to progressively decrease.

The regression analysis revealed that the extraction process seemed to be biphasic, i.e. having two phases, each with its own rate of exponential decay This could suggest that L-Dopa molecules near the particle surfaces are more easily extracted (early phase) than the L-Dopa molecules trapped deep within the particle interior that must diffuse through the solid matrix of the bean structure in order to reach the surface once the surface regions become depleted (predominant phase). Linear extrapolation of the predominant phase curves can be used to predict the time required to reach the safe target level for any particle size.

Effect of water circulation

In order to study the effect of circulation, two additional experiments were carried out with 1 mm particles using room temperature tap water (pH 7.0, 20°C), but with and without circulation (i.e., stagnant water). These data show that the circulation rate used in this study had no effect on extraction rate. Use of water bath circulation in this study was originally done to achieve uniform temperature distribution in the water bath. It was also expected to induce relative fluid velocity at the water-particle surface interface, but the rate was evidently much too slow to induce any significant relative velocity at the particle interface. Therefore, experiments designed with significant relative velocity should be planned for future study. Note also the wide variability in results from replicate experiments with circulating bath water. One experiment resulted in nearly twice as much residual L-Dopa as the other after 24 h.

Effect of water temperature

Clearly, raising the water temperature improves the extraction rate dramatically. The extraction curves suggest that the safe target level with 1 mm particles can be reached within 13 h at 40°C and 3 h at 66°C using neutral pH tap water. At the highest temperature, the bath water colour turned black during the extraction, which did not occur at lower temperatures. The changes in L-Dopa content upon soaking at different water temperatures was studied by Bressani *et al.* (2003). Soaking the seeds for 96.5 h at 22°C resulted in 30% loss of L-Dopa. The L-Dopa retension was decreased to 51% at 45°C and to 27% at 66°C after 96.5 h soaking.

Although water uptake did not increase significantly with prolonged soaking time or with higher temperature, L-Dopa dropped significantly as water temperature increased. It is notable that there was no change in water during the complete soaking period at the higher temperatures. It seems that water temperature therefore plays a significant role in L-Dopa removal from the velvet beans. Projection by using mathematical relationship linear equation to 0% L-Dopa content would indicate that a temperature of 95°C for 96.5 h would be required, assuming the solubility of L-Dopa will increased with the temperature. However, soaking time for 96.5 h is not attractive for standard food preparations.

Effect of water to seed ratio

Myhrman (2002) studied the relationship between the extraction effectiveness and the ratio of seeds to water and found that the effectiveness of extraction increases as the mass of beans in the quantity of water is decreased. In a series of experiments, the seeds to water ratio (1 g of seeds per 40 ml of water) of 8:40, 6:40, 4:40, 2:40 and 1:40 exhibits L-Dopa residual level of 0.82%, 0.66%, 0.41%, 0.093% and 0.025%, respectively. From this study it is evident that any decrease in the ratio of seeds to water will result in an increase in the effectiveness of extraction of L-Dopa.

Effect of change of water

L-Dopa level of velvet beans soaked in water at 60°C with periodical water change was reduced upto 30% in 48 h (Bressani *et al.* 2003). Based on this study, it is suggested that soaking water should be at a higher temperature and often changed for better L-Dopa removal from the velvet bean seeds.

Effect of acid/alkaline range pH

Residual L-Dopa levels in response to adjusting pH in the acid range with water at room temperature. These results also show dramatic increase in extraction rates with 1 mm particles in acid solution at pH 3, similar to increasing temperature to 66°C with pH neutral tap water. Note that even at just pH 5.0, safe target levels can be reached within 8 h if

water is heated to only 40°C.

Residual L-Dopa levels in response to adjusting pH in the alkaline range with water at room temperature are increasing alkalinity of the water to pH 11.0 also showed equally dramatic decline in residual L-Dopa. However, the bath water immediately turned totally black during these extraction experiments at high pH. This near instantaneous colour change suggests that the decline in residual L-Dopa levels observed at high pH may be the result of a chemical conversion of L-Dopa to melanin, rather than actual extraction into the water. Samples of water extract at pH 7.0 and pH 11.0 were assayed for residual L-Dopa. Results showed expected concentration levels at pH 7.0 but none detected at pH 11.0, which further suggests chemical conversion to melanin. No attempt was made to detect melanin within the bean particles, which could also be a topic for further study.

L-Dopa is readily soluble in dilute solutions of hydrochloric acid as reported in the Merck index (1983). In fact many traditional assay techniques for determining the level of L-Dopa in a sample begin by performing a total extraction in hydrochloric acid (Daxenbichler *et al.* 1971). Given this fact, a study of extractions using mildly acidic solutions made with food-grade acid ingredients may have merit if water extraction is impractical. Also, because of potential protein denaturation at low pH, the effect of acidity on the protein quality will have to be studied if acid solvents are to be considered. Although no information was found in published literature concerning solubility of L-Dopa in alkaline solutions, some evidence is reported in Diallo *et al.* (2002). Their work reported that a calcium hydroxide solution appeared to outperform water as a potential solvent for removing L-Dopa from *Mucuna* seed, that would merit further study.

Combination of treatments

To determine the level of reduction of L-Dopa Diallo *et al.* (2002) soaked the velvet bean seeds in distilled water for 48 h with a change of fresh water for every 8-12 h. It is then boiled for 45-60 min, their seed coat was removed, they were roasted for 20 min and finally cooked for 30-60 min. A combination of all the treatments (soaking, roasting and boiling) removed the most of the L-Dopa and leaving the seeds with L-Dopa levels as low as 0.045%. Roasting prior to soaking and cooking seems to more effective. Roasting the seeds for 20-30 min with seed coat intact reduced the L-Dopa content by 30-40%. Among the treatments, the best method as reported by Diallo *et al.* (2002) was to roast the seeds for 20-30 min, crack them, soak them in water for 48 h with changing the fresh water for every 8-12 h and then cook them for 2 h, which lowers the L-Dopa level to below 0.1%.

Myhrman (2002) developed a combination of processing methods to remove the L-Dopa from the velvet bean seeds. The seeds were cracked by hand and cut the bean into pieces, soak them overnight and the seeds were washed with water and the seed coat was removed. Then the seeds were boiled in fresh water for 45 min and allow the pot to cool for 4-6 h and the seeds were washed again soaked for overnight. Then the seeds were washed and dried in sunlight and grind the seeds into flour. By this method, the L-Dopa level in the velvet bean was reduced from 6.4% to 0.42%. Bhat *et al.* (2007) studied the effect of ionizing radiation on the levels of L-Dopa in the seeds of *M. pruriens*. The L-Dopa concentration showed a dose-dependant decline when the velvet bean seed materials were exposed to doses of 2.5, 5.0, 7.5, 10, 15 and 30 kGy gamma radiation. Gurumoorthi *et al.* (2007) also noticed the significant reduction in the levels of L-Dopa during gamma radiation treatment at 5kGy.

Raw *Mucuna* contained 6.36% of L-Dopa by the weight. Boiling for 45 min followed by dehulling reduced level by 25.89%. Soaking the cooked and dehulled bean for 12 h reduced L-Dopa by 63.88% and a further 12 h soaking after removal of soaking water reduced it by 78.63%. Reduction

of L-Dopa took place mainly through leaching. Some enzymatic activity may also take place during the second soaking period as shown by the formation of foam at the top of water. Further cooking of the velvet beans for 45 min eliminated 89.95% of the toxic principle (Egounlety 2003). Germination of the velvet bean seeds for 7 days followed by dehulling and boiling for 90 min reduced 42% of L-Dopa (Wanjekeche et al. 2003). Ferreira et al. (2003) repoted the combination of treatments such as roasting followed by grinding, grinding followed by roasting, soaking, roasting followed by grinding and soaking, heat drying followed by grinding reduced the L-Dopa content of velvet bean seeds from 5.52% to 5.44%, 5.51%, 4.92% and 5.37%, respectively.

Effect of combination of different processing methods on the L-Dopa content was examined by many workers. Versteeg et al. (1998) and Lorenzetti et al. (1998) reported the treatment of combination of roasting, seed-coat removal, soaking, and boiling procedures to minimize the L-Dopa content of Mucuna. Recipes prepared with roasted, soaked and boiled Mucuna contained as little as 0.8% L-Dopa, in contrast to levels of 4.67-6.15% in untreated seeds. It is clear therefore that these processing methods removed most of the L-Dopa from Mucuna. The low L-Dopa content was further diluted to 0.2% by mixing one portion of Mucuna flour with three portions of cereal (e.g., maize, sorghum) or cassava flour.

Immersion of seeds in natural running water

This method should be considered in all situations where access to a nearby flowing stream or river is possible. The process consists of submerging burlap-type bags (or similar strong and porous sack) filled with cracked and de-hulled Mucuna beans into a flowing river or stream. This method brings a constant supply of continuous-flowing pure solvent in contact with the bean particles with little energy, labor, or equipment requirement. The flowing water past the sack imparts relative velocity at the particle/solvent interface (agitation), and the constant supply of fresh water maintains a maximum concentration gradient for effective extraction (Balaban and Teixeira 2002).

Overflowing trough and rake

It is most appropriate when access to a river or stream is not practical, but fresh water is still in abundant supply. A simple watering trough can be used to hold the batch of cracked de-hulled beans to be processed. It is kept filled to overflowing with a constant supply of fresh water as the batch of beans is stirred with a rake to provide agitation. When extraction is completed, the trough is drained, and the beans recovered for safe use (Balaban and Teixeira 2002).

Plant breeding studies

One possible approach to reduce the L-Dopa content in velvet bean seeds by breeding programmes. For food/feed use, the target level of L-Dopa would be low and if Mucuna is to be grown as a pharmaceutical source of L-Dopa, the target level would be high while the L-Dopa content should be low in case if the velvet beans are used as food/feed. A program in the southeastern United States, early in the 20th century, used conventional hybridization to develop a number of Mucuna varieties that were widely distributed and grown as a green manure/cover crop. The levels of L-Dopa were reduced during the breeding and selection process, but other important plant characteristics such as crop cycle and maturity were also altered, and no reports of studies using near-isogenic lines (same genotype except for L-Dopa content) are available to prove conclusively that the effects are not pleiotropic.

When approaching the L-Dopa problem through plant breeding programme, the other concerns in reducing the L-Dopa content should also be considered. If allelopathy is the agency by which Mucuna is effective in controlling weeds, it will be important to focus breeding or other genetic modification efforts on the seeds only, as opposed to reducing L-Dopa throughout the entire plant. Resistance to bruchid beetles, which prey on seeds of many other legumes, is an important issue if breeding is to focus on reducing L-Dopa in Mucuna seeds. As Lorenzetti et al. (1998) pointed out, a reduction in L-Dopa might results in an increase in predation on Mucuna seeds by a number of insects. The implications for Mucuna breeding are that the genes controlling L-Dopa may have pleiotropic effects on other properties that directly or indirectly affect Mucuna's resistance to diseases.

The L-Dopa level may not be the only factor affecting the plant's disease resistance and it will be important to look for these effects and genetic interactions in breeding Mucuna. It would be important to make sure that in breeding for lower L-Dopa content, properties of Mucuna affecting its desirability as a nematode suppressant would remain unaffected or would be improved. Because Mucuna tolerates a wide range of soil acidity, moisture, fertility, and physical properties, is a strong nitrogen fixer, and has an aggressive growth habit and high level of productivity of vegetative matter, it is an excellent means of improving soil and protecting it from erosion (Hairiah et al. 1993; Sanginga et al. 1996; Buckles et al. 1998). If reducing L-Dopa conferred a general reduction of fitness, and this reduced vegetative growth or nitrogen metabolism, the plant would be less useful. Reducing the level of L-Dopa by itself, with no other genetic modifications might, in the short term, actually confer a fitness disadvantage to the plant. However, if the medium- to long-term goal, would be to reduce L-Dopa levels in the seeds only, and breed the plant for other forms of resistance to pests and diseases, the crop plant might ultimately turn out to have roughly the same or even a higher level of fitness.

Breeding efforts reported by Krishnamurthy et al. (1996) for the purpose of increasing levels of L-Dopa for pharmaceutical use, showed that seeds of a wild, parental line of Mucuna had not only an L-Dopa content of 4.96%, but also the presence of itching trichomes on the seed pods, making harvest difficult. Some domesticated parental lines had fewer or no trichomes, but also had lower L-Dopa contents (3.16-4.01%). Two parental lines with low L-Dopa content, however, did have trichomes. Crosses were developed with higher L-Dopa content and few or no trichomes. This suggested that the genes for L-Dopa content and itching trichomes are inherited independently, so that it should also be possible to develop lines with lower L-Dopa content and more protective trichomes. The technology, which involves specific manipulation of both genes and their promoters, depends on "knockout" (or antisense) control of gene expression that could be placed "up stream" or "down stream" in the metabolic pathway for L-Dopa production. Of course, the potential application of such a technology to the goal of reducing L-Dopa levels in a tissue-specific manner would require a substantial research effort. Ideally, farmers would prefer a variety in which the production or non-production of L-Dopa does not depend on externally purchased chemicals (or other production inputs) to activate or de-activate the plant's metabolic machinery. Hence, this genetic engineering approach may be attempted to eliminate the high content of L-Dopa in velvet bean seeds in future.

CONCLUSION

The available reports shows that L-Dopa is present at high concentrations in the seeds of Mucuna pruriens (velvet beans) and many factors were found to influence their levels. The recently developed analytical methods were found to be rapid and more sensitive in determining the L-Dopa content, which will be useful for routine analysis in future. However, methodologies should be developed to detect their present in animal tissues including milk. The L-

Dopa compound was found to possess good antioxidant potential under *in vitro* studies. However, their presence in the diet above the appropriate level would result in poor growth performance of experimental animals, particularly fishes and poultry. Hence, extensive studies should be undertaken to evaluate the health impacts of L-Dopa for the consuming animals including human beings and the safe level of L-Dopa in the diet for all the animals should be determined, because it varies with species to species. Further, although, many processing methods were devised to reduce the L-Dopa content in velvet bean seeds, since it is a single compound, it may either oxidized or converted into its derivatives, which again undesirable for consumption. Hence, some other novel biotechnological food processing should be targeted to complete degradation of this compound, which will enhance the utilization of velvet beans as potential alternative protein source for both human beings and animals.

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REFERENCES

Afolabi OA, Oshuntogun BA, Adewusi SR, Fapojuwo OO, Ayorinde FO, Grissom FE, Oke OL (1985) Preliminary nutritional and chemical evaluation of raw seeds from *Mucuna solanei*- An under utilized food source. *Journal of Agricultural and Food Chemistry* **33**, 122-124

Agil A, Duran R, Barrero F, Marles B, Arauzo M, Alba F, Miranda MT, Prieto I, Ramirez M, Vives F (2006) Plasma lipid peroxidation in sporadic Parkinson's disease. Role of L-Dopa. *Journal of Neurological Sciences* **240**, 31-36

Aguiyi JC, Igweh AC, Egesie UG, Leoncini R (1999) Studies on possible protection against snake venom using *Mucuna pruriens* protein immunization. *Fitoterapia* **70**, 21-24

Amin KMY, Khan MN, Zillur-Rehman S, Khan NA (1996) Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats. *Fitoterapia* **LXVII**, 53-56

Arnow LE (1937) Colorimetric determination of the components of 3,4-dihydroxyphenylalanine-tyrosine mixture. *The Journal of Biological Chemistry* **118**, 531-537

Ates S, Cortentioglu E, Bayrakhor E, Mehmetoglu U (2007) Production of L-Dopa using Cu-alginate gel immobilized tyrosinase in a batch and packed bed reactor. *Enzyme and Microbial Technology* **40**, 683-687

Balaban MO, Teixeira AA (2002) Potential home and industrial process treatments to reduce L-Dopa in *Mucuna* bean. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 339-351. Available online: www.cidicco.hn.

Barbeau A, McDowell FH (1970) L-Dopa *and Parkinsonism*, F.A. Davis Company, Philadelphia, PA, USA, pp 403-435

Bell EA, Janzen DH (1971) Medical and ecological considerations of L-Dopa and 5-HTP in seeds. *Nature* **299**, 136-137

Bell EA, Nulu JR, Cone C (1971) L-Dopa and L-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, a new imino acid, from seeds of *Mucuna inutisiana*. *Phytochemistry* **10**, 2191-2194

Bergamini MF, Santos AL, Stradiotto NR, Zanoni MVB (2005) A disposable electrochemical sensor for the rapid determination of levodopa. *Journal of Pharmaceutical and Biomedical Analysis* **39**, 54-59

Berner DK, Killani AS, Aigbokhan E, Couper DC (1992) *Macrophomina phaseolina* on the tropical cover crop *Mucuna pruriens* var. *utilis*. *Plant Disease* **76**, 1283-1284

Bhat R, Sridhar KR, Tomita-Yokotani K (2007) Effect of ionizing radiation on antinutritional features of velvet bean seeds (*Mucuna pruriens*). *Food Chemistry* **103**, 860-866

Blanchart E, Villenaue C, Viallatoux A, Barthes B, Girardin C, Azontonde A, Feller C (2006) Long-term effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on the communities of soil microfauna and nematofauna under maize cultivation in Southern Benin. *European Journal of Soil Biology* **42**, 136-144

Brain KR (1976) Accumulation of L-Dopa in cultures from *Mucuna pruriens*. *Plant Science Letters* **7**, 157-161

Bressani R (2002) Factors influencing nutritive value in food grain legumes: *Mucuna* compared to other grain legumes. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 164-188. Available online: www.cidicco.hn

Bressani R, Lau M, Silvia Vargas M (2003) Protein and cooking quality and residual content of dehydroxyphenylalanine and of trypsin inhibitors of processed mucuna beans (*Mucuna* spp). *Tropical Subtropical Agroecosystems* **1**, 197-212

Buckles D (1995) Velvet bean: A "new" plant with a history. *Economic Botany* **49**, 13-25

Buckles D, Triomphe B, Sain G (1998) Cover Crops in Hillside Agriculture: Farmer Innovation with *Mucuna*. IDRC and CIMMYT, Ottawa, Canada, pp 123-126

Budavaris S, O'Neil M, Smith A, Heckelman P (1989) *The Merck Index* (11th Edn) Merck & Co., Inc. New Jersey, US, pp 217-225

Burbano C, Cuadrado C, Muzquiz M, Cubero JI (1995) Variation of favism inducing factors (vicine, convicine and L-Dopa) during pod development in *Vicia faba* L. *Plant Foods for Human Nutrition* **47**, 265-274

Burgos A, Matamoros I, Toro E (2002) Evaluation of velvet bean (*Mucuna pruriens*) meal and *Enterolobium ciclocarpum* fruit meal as replacements for soybean meal in diets for dual-purpose cows. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 228-237. Available online: www.cidicco.hn

Camara A, Toupou K, Diallo D, Berhe T (2003) Studies on *Mucuna* as poultry and pig feed in the republic of Guinea. *Tropical Subtropical Agroecosystems* **1**, 247-252

Cannazza G, Stefamo AD, Mosciatti B, Braghiroli D, Baraidi M, Pinnen F, Sozio P, Benutti C, Parenti C (2005) Detection of levodopa, dopamine and its metabolites in rat striatum dialysates following peripheral administration of L-Dopa prodrug by means of HPLC-EC. *Journal of Pharmaceutical and Biomedical Analysis* **36**, 1079-1084

Capo-chichi LJA, Weaver DB, Morton CM (2003) The use of molecular markers to study genetic diversity in *Mucuna*. *Tropical and Subtropical Agroecosystems* **1**, 309-318

Carew LB, Alster FA, Gernat AG (1998a) Blood chemistry including cholesterol, glucose and thyroid hormones of broilers fed raw velvet beans (*Mucuna pruriens*). *Journal of Animal Science* **76**, 82-85

Carew LB, Alster FA, Gernat AG (1998b) Consumption of raw velvet beans (*Mucuna pruriens*) alters organ weights and intestinal lengths in broilers. *Poultry Science* **77**, 56-57

Carew LB, Hardy D, Weis J, Alster F, Mischler SA, Gernat A, Zakrzewska EI (2003) Heating raw velvet beans (*Mucuna pruriens*) reverses some antinutritional effects on organ growth, blood chemistry, and organ histology in growing chickens. *Tropical Subtropical Agroecosystems* **1**, 267-276

Carew LB, Valverde MT, Zakrzewska EI, Alster FA, Gernat AG (2002) Raw Velvet beans (*Mucuna pruriens*) and L-Dopa have differing effects on organ growth and blood chemistry when fed to chickens. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 272-287. Available online: www.cidicco.hn

Carlini R, Udedibie B (1997) Comparative effects of processing methods on hemagglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds. *Journal of Agricultural and Food Chemistry* **45**, 4372-4377

Carsky RJ, Becker M, Hauser S (2001) *Mucuna* cover crop fallow systems: potential and limitations. In: Tian G, Ishida F, Keatinge D (Eds) *Sustaining Soil Fertility in West Africa*, SSSA Special Publication no. 58, Soil Science Society of America and American Society of Agronomy, Madison, WI, USA, pp 111-135

Carsky RJ, Tarawali SA, Becker M, Chikoye D, Tian G, Sanginga N (1998) *Mucuna* – herbaceous cover legume with potential for multiple uses. Resource and Crop Management Research Monograph 25. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp 12-25

Castillo-Caamal JB, Jiménez-Osornio JJ, López-Pérez A, Aguilar-Cordero W, Castillo-Caamal AM (2003) Feeding *mucuna* beans to small ruminants of Mayan farmers in the Yucatan Peninsula, Mexico. *Tropical Subtropical Agroecosystems* **1**, 113-118

Chen AS, Wei C, Rolle RS, Otwell WS, Balaban MO, Marshall MR (1991) Inhibitory effect of kojic acid on some plant and crustacean polyphenol oxidases. *Journal of Agricultural and Food Chemistry* **39**, 1396-1401

Cook DG, Klawans HL (1985) Levodopa during pregnancy. *Clinical Neuropharmacology* **8**, 93-95

D'Mello JPF, Walker AG (1991) Detoxification of jack beans (*Canavalia ensiformis*): Studies with young chicks. *Animal Feed Science and Technology* **33**, 117-127

Daxenbichler ME, van Etten CH, Earle FR, Tallent WH (1972) L-Dopa recovery from *Mucuna* seed. *Journal of Agricultural and Food Chemistry* **20**, 1046-1048

Daxenbichler ME, van Etten CH, Hallinan EA, Earle FR, Barclay AS

(1971) Seeds as sources of L-Dopa. *Journal of Medical Chemistry* **14**, 463-465

del Carmen J, Gernat AG, Myhrman R, Carew LB (1999) Evaluation of raw and heated velvet beans (*Mucuna pruriens*) as feed ingredients for broilers. *Poultry Science* **78**, 866-872

Diallo OK, Berhe T (2003) Processing of *Mucuna* for human food in the Republic of Guinea. *Tropical Subtropical Agroecosystems* **1**, 193-196

Diallo OK, Kante S, Myhrman R, Soumah M, Cisse NY, Berhe T (2002) Increasing farmer adoption of *Mucuna pruriens* as human food and animal feed in the republic of Guinea. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 60-72. Available online: www.cidicco.hn

D'Mello JPF, Duffus CM, Duffus JH (1991) Non-protein toxic amino acids In: D'Mello JPF, Duffus CM, Duffus JH (Eds) *Toxic Substances in Crop Plants*, Royal Society of Chemistry, Cambridge, London, pp 285-327

Dollery C (1999) *Therapeutic Drugs* (2nd Edn), Livingstone Churchill, New York, pp 39-43

Duffus CM, Duffus JH (1991) Antinutritional compounds of legume seeds. In: D'Mello JPF, Duffus CM, Duffus JH (Eds) *Toxic Substances in Crop Plants*, Thomas Graham House, Science Park, Cambridge, Royal Society of Chemistry, Cambridge, London, pp 1-21

Duh PD, Yen WJ, Du PC, Yen GC (1997) Antioxidant activity of mung bean hulls. *Journal of the American Oil Chemists' Society* **74**, 1059-1063

Duke JA (1981) *Handbook of Legumes of World Economic Importance*, Plenum Press, New York, USA, pp 170-173

Egounlety M (2003) processing of velvet bean (*Mucuna pruriens* var *utilis*) by fermentation. *Tropical Subtropical Agroecosystems* **1**, 173-182

Elwan MA, Sakuragawa H (2007) Uptake and decarboxylation of L-2,4-Dihydroxyphenylalanine in cultured monkey placenta amniotic epithelial cells. *Placenta* **28**, 245-248

Eskin NAM, Wiebe S (1983) Changes in phytase activity and phytate activity and phytate during germination of two faba bean cultivars. *Journal of Food Science* **48**, 270-271

Eskow KL, Gupta V, Alam S, Park JY, Bishop C (2007) The partial 5-HTIA agonist buspirone reduces the expression and development of L-Dopa-induced dyskinesia in rats and improves L-Dopa efficacy. *Pharmacology Biochemistry and Behaviour* **87**, 306-314

Evans CS, Shah AJ, Adlard MW, Arce MLR (1993) Non-protein amino acids in seeds of neotropical species of *Acacia*. *Phytochemistry* **32**, 123-126

Ezeagu IE, Sridevi, Haridas Rao P, Harandranath, Appu Rao AG, Gowda LR, Tarawale G (2002) Prospects for incorporation of defatted *Mucuna* flour in biscuit formulation. *Journal of Food Science and Technology* **38**, 435-438

Ferriera HA, Peña BK, Gernat AG, Carew LB, Matamoros IA (2003) Evaluation of different processing methods of velvet bean (*Mucuna pruriens*) for use as a feed ingredient for broilers. *Tropical Subtropical Agroecosystems* **1**, 277-286

Finco DR (1997) Kidney function. In: Kaneko JJ, Harvey JW, Bruss ML (Eds) *Clinical Biochemistry of Domestic Animals*, Academic Press, Boston, MA, pp 470-473

Flores LM, Esnaola A, Myhrman R (2002) Growth of pigs fed diets with *Mucuna* bean flour (*Mucuna pruriens*) compared to soybean meal. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 288-305. Available online: www.cidicco.hn

Fowden L (1976) Non-protein nitrogen compounds: Toxicity and antagonistic action in relation to amino acid and protein synthesis. In: Norton G (Ed) *Plant Proteins*, Butterworths, London, Boston, pp 109-115

Fujii Y, Shibuya T, Yasuda T (1992) Allelopathy of velvet bean: Its discrimination and identification of L-Dopa as a candidate of allelopathic substances. *Japanese Journal of Agricultural Research Quarterly* **25**, 238-247

Gilbert RA (2002) *Mucuna pruriens* in Malawi: A promising legume with a troubled history. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 48-59. Available online: www.cidicco.hn

Gliessman SR (1983) Allelopathic interactions in crop-weed mixtures: applications for weed management. *Journal of Chemical Ecology* **9**, 991-999

Gongke Z, Yingzhen K, Kairong C, Zhixiao L, Yafu W (2001) Hydroxyl radical scavenging activity of β-N-oxalyl-L-α,β-diaminopropionic acid. *Phytochemistry* **58**, 759-762

Grover JK, Yadav S, Vats V (2002) Medicinal plants of India with anti-diabetic potential. *Journal of Ethnopharmacology* **81**, 81-100

Guerranti R, Aguiyi JC, Errico E, Pargani R, Marinello E (2001) Effects of *Mucuna pruriens* extract on activation of prothrombin by *Echis carinatus* venome. *Journal of Ethnopharmacology* **75**, 175-180

Gurumoorthi P, Janardhanan K, Myhrman R (2007) Effect of differential processing methods on L-Dopa and protein quality in velvet bean, an underutilized pulse. *LWT - Food Science and Technology*, in press

Gurumoorthi P, Senthil kumar S, Vadivel V, Janardhanan K (2003) Studies on agrobotanical characters of different accessions of velvet bean collected from Western Ghats, South India. *Tropical Subtropical Agroecosystems* **2**, 105-115

Hachinohe M, Matsumoto H (2007) Involvement of melanin synthesis and reactive oxygen species in phytotoxic action of L-Dopa in carrot cells. *Crop protection* **26**, 294-298

Hairiah K, Van Noordwijk M, Stulen I, Meijboom FW, Kuiper PJC (1993) Phosphate nutrition effects on aluminum avoidance of *Mucuna pruriens* var. *utilis*. *Environmental Experimental Botany* **33**, 75-83

Harms RH, Simpson CF, Waldroup PW (1961) Influence of feeding various levels of velvet beans to chicks and laying hens. *Journal of Nutrition* **75**, 127-134

Hasan BA, Khalat KD, Guardia MDL (1995) Flow analysis-spectrophotometric determination of L-Dopa in pharmaceutical formulations by reaction with *p*-aminophenol. *Talanta* **42**, 627-633

He WW, Zhou XW, Lu JG (2006) Simultaneous determination of benserazide and levodopa by capillary electrophoresis-chemiluminescence using an improved interface. *Journal of Chromatography A* **1131**, 289-292

Higasa S, Negishi Y, Adoyagi Y, Sugahara T (1996) Changes in free amino acids of tempe during preparation with velvet beans (*Mucuna pruriens*). *Journal of the Japanese Society of Food Science and Technology* **43**, 188-193

Ho PY, Chuang GS, Li HY (2005) Computational steady states for enzymic production of L-Dopa in an isothermal CSTR. *Process Biochemistry* **40**, 469-478

Houghton PJ, Skari KP (1994) The effect on blood clotting of some west African plants used against snakebite. *Journal of Ethnopharmacology* **44**, 99-108

Huang SY, Chau SN (2006) Elucidation of the effects of nitrogen source on the proliferation of transformed hairy roots and secondary metabolite productivity in a mist trickling reactor by redox potential measurement. *Enzyme and Microbial Technology* **38**, 803-813

Huang SY, Chen SY (1998) Efficient L-Dopa production by *Stizolobium hassjoo* cell culture in a two stage configuration. *Journal of Biotechnology* **62**, 95-103

Huang SY, Chen SY, Wu KL, Taung WT (1995) Strategy for inducing pertinent cell line and optimization of the medium for *Stizolobium hassjoo* producing L-Dopa. *Journal of Fermentation and Bioengineering* **79**, 342-347

Huang SY, Hung C, Chou S (2004) Innovative strategies for operation of mist trickling reactors for enhanced hairy root proliferation and secondary metabolite productivity. *Enzyme and Microbial Technology* **35**, 22-32

Huang SY, Shen Y, Chau H (2002) Development of a bioreactor operation strategy for L-Dopa production using *Stizolobium hassjoo* suspension culture. *Enzyme and Microbial Technology* **30**, 779-791

Hurrell RF, Finot PA (1984) Nutritional consequences of the reactions between proteins and oxidized polyphenolic acids. *Advanced Experiments in Medicinal Biology* **177**, 423-435

Hussain G, Manyam BV (1997) *Mucuna pruriens* proves more effective than L-Dopa in Parkinson's Disease animal model. *Phytotherapy Research* **11**, 419-423

Infante ME, Perez AM, Simao MR, Manda F, Baquete EF, Fernandez AM (1990) Outbreak of acute psychosis attributed to *Mucuna pruriens*. *The Lancet* **336**, 1129-1130

Iversen SD, Iversen LL (2007) Dopamine – 50 years in perspective. *Trends in Neurosciences* **30**, 188-193

Janardhanan K, Gurumoorthi P, Pugalenthi M (2003b) Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens* var.*utilis* Part I. The effect of processing methods on the contents of L-Dopa, phytic acid, and oligosacaharides. *Tropical Subtropical Agroecosystems* **1**, 141-152

Janardhanan K, Lakshmanan KK (1985) Studies on the pulse, *Mucuna utilis*: Chemical composition and antinutritional factors. *Journal of Food Science and Technology* **22**, 369-371

Janardhanan K, Vadivel V, Pugalenthi M (2003a) Biodiversity in Indian under-exploited/tribal pulses. In: Jaiwal PK, Singh RP (Eds) *Improvement Strategies for Leguminosae Biotechnology*, Kluwer Academic Publishers, UK, pp 353-405

Jayaweera DMA (1981) *Medicinal Plants Used in Ceylon*, National Science Council of Sri Lanka, Colombo, Sri Lanka, pp 312-345

Jones TC, Hunt RD (1983) Patologia veterinaria. Hepatitis tóxica aguda. Trans. by Carlos Lightowler (5th Edn) Hemisferio Surgery Uruguay **3**, 1438-1440

Kageyama T, Nakamura M, Matsuo A, Yamasaki Y, Takakura Y, Hashida M, Kanai Y, Naito M, Tsuruo T, Minato N, Shimohama S (2000) Complex transports L-Dopa across the blood-brain barrier. *Brain Research* **879**, 115-121

Kaizzi CK, Sali HS, Viek PLG (2006) Differential use and benefits of velvet bean (*Mucuna pruriens* var. *utilis*) and N fertilizers in maize production in contrasting agro-ecological zones of Uganda. *Agricultural Systems* **88**, 44-60

Kawazoe T, Tsuge H, Imagawa T, Aki K, Kuramitsu S, Furkui K (2007) Structural basis of D-Dopa oxidation by D-amino acid oxidase: Alternative pathway for dopamine biosynthesis. *Biochemical and Biophysical Research Communications* **355**, 385-391

Keagy EM, Carew LB, Alster FA, Tyzbir RS (1987) Thyroid function, energy balance, body composition and organ growth in protein-deficient chicks. *Journal of Nutrition* **117**, 1532-1540

Kiro A, Zahavi I, Mercus H, Dinari G (1992) L-Dopa is protective against indomethcin-induced small intestinal ulceration in the rat: Possible role of a

α-2-adreagic mechanism. *Life Sciences* **51**, 1151-1156

Kloepper J, Rodriguez-Kabana R, McInroy JA, Collins DJ (1991) Analysis of populations and physiological characterization of microorganisms in rhizospheres of plants with antagonistic properties to phytopathogenic nematodes. *Plant and Soil* **136**, 95-102

Korycka-Dahl M, Richardson M (1978) Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acids. *Journal of Dairy Science* **61**, 400-407

Koyanagi T, Katayama T, Suzuki H, Nakazawa H, Yokoziki K, Kumagai H (2005) Effective production of L-3,4-Dihydroxyphenylalanine (L-Dopa) with *Erwinia herbicola* cells carrying a mutant transcriptional regulator TyrR. *Journal of Biotechnology* **115**, 303-306

Krishnamurthy R, Suvagia P, Pathak JM, Parikh KM, Bhikne NS (1996) Selection of *Mucuna* lines for higher L-Dopa and improved yield through conventional breeding. *Bundesanstalt für Zuchtungsforschung an Kulturpflanzen* **2**, 231-234

Larher I, Gerard J, Gerant-Sauvage D, Hamelin J, Briens M (1984) An assessment of the potential of *Vicia faba* minor in the storage of the L-form of 3,4-dihydroxyphenylalanine. *Journal of Plant Physiology* **116**, 71-80

Latellier S, Garnier JP, Spy J, Stoitchkov K, Le Bricon T, Baccard M, Revol M, Kerneis Y, Bousquet B (1999) Development of metastases in malignant melanoma is associated with an increase in the plasma L-Dopa/L-tyrosine ratio. *Melanoma Research* **9**, 389-394

Laurent L, Livesey J, Arnason JT, Bruneau A (2002) Variation in L-Dopa concentration in accessions of *Mucuna pruriens* (L.) DC. and in *Mucuna brachycarpa* Rech. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 352- 375. Available online: www.cidicco.hn

Lee KG, Mitchell AE, Shibamoto T (2000) Determination of antioxidant properties of aroma extracts from various beans. *Journal of Agricultural and Food Chemistry* **48**, 4817-4820

Leff SE, Rendahl KG, Spratt SK, Kang UJ, Mandel RJ (1998) *In vivo* L-Dopa production by genetically modified primary rat fibroblast or 9Lgliosarcoma cell grafts via coexpression of GTP cyclohydrolase I with tyrosine hydroxylase. *Experimental Neurology* **151**, 249-264

Lennernas H, Nilsson D, Aquilonius SM, Paalzow LK, Ahrenstedt O, Knutsson L, Hallgren R (1992) Mechanistic evaluation of the intestinal absorption of L-Dopa in humans using a newly developed *in vivo* intestinal perfusion instrument Loc-L-Gut. *Journal of Controlled Release* **21**, 204-205

Liu L, McClure JW (1995) Effects of UV-B on activities of enzymes of secondary phenolic metabolism in barley primary leaves. *Physiologia Plantarum* **93**, 734-739

Lobo Burle M, Suhet AR, Pereira J, Resck DVS, Peres JRR, Cravo MS, Bowen W, Bouldin DR, Lathwell DJ (1992) Legume green manures: their dry-season survival and the effect on succeeding maize crop. Soil Management CRSP Bulletin No. 92-04. Soil Management Collaborative Research Support Program. Raleigh, North Carolina, pp 30-34

Longo R, Castellani A, Sberze P, Tibolla M (1974) Distribution of L-Dopa and related amino acid in *Vicia*. *Phytochemistry* **13**, 167-171

Lorenzetti F, MacIsaac S, Arnason JT, Awang DVC, Buckles D (1998) The phytochemistry, toxicology and processing potential of the cover crop velvet bean (Cow(h)age, Cowitch) (*Mucuna* Adans. spp., Fabaceae). In: Buckles D, Etèka A, Osiname O, Galiba M, Galiano N (Eds) *Cover Crops in West Africa. Contributing to Sustainable Agriculture*, IDRC, Ottawa, Canada, pp 67-84

Lumeij JT (1997) Avian clinical biochemistry. In: Kaneko JJ, Harvey JW, Bruss ML (Eds) *Clinical Biochemistry of Domestic Animals*, Academic Press, Boston, MA, pp 864-873

Mager J, Chevion M, Glaser G (1980) Favism. In: Liener IE (Ed) *Toxic Constituents of Plant Foodstuffs*, Academic Press, New York, pp 266-288

Maggi N, Cometti A (1972) Colorimeteric assay of levodopa. *Journal of Pharmaceutical Science* **61**, 924-927

Makker HPS, Becker K (1998) Plant toxins and detoxification methods to improve feed quality of tropical seeds- Review. In: *Pre-conference Symposium II on Management of feed resources and animal waste for sustainable animal production in Asia–Pacific region beyond 2000*; 8th world Conference on Animal production. June 28, 1998. Seoul National University, Seoul, Korea, pp 467-480

Marquardt RR, Frohlich AA (1981) Rapid reverse-phase high performance liquid chromatographic method for the quantitation of vicine, convicine and related compounds. *Journal of Chromatography A* **208**, 373-379

Mary Josephine R, Janardhanan K (1992) Studies on chemical composition and antinutritional factors in three germplasm seed materials of the tribal pulse, *Mucuna pruriens* (L.) DC. *Food Chemistry* **43**, 13-18

Mashkoor M, Siddiqui MA, Ahmad A (1990) Antagonistic plants. In: Jairajpuri MS, Mashkoor M, Ahmad I (Eds) *Nematode Bio-Control: Aspects and Prospects*, New Age International, New Delhi, India, pp 41-50

Matenga VR, Ngongoni NT, Titterton M, Maasdorp BV (2003) *Mucuna* seed as a feed ingredient for small ruminants and effect of ensiling on its nutritive value. *Tropical Sub-tropical Agroecosystems* **1**, 97-106

Mendoza-Castillo H, Castillo-Caamal JB, Ayala-Burgos A (2003) Impact of *Mucuna* bean (*Mucuna* spp.) supplementation on milk production of goats. *Tropical Subtropical Agroecosystems* **1**, 87-92

Muiswinkel FL, Riemers FM, Peters GJ, Latleur VM, Siegel D, Jongenelen CAM, Drukarch B (2000) L-Dopa stimulates expression of the antioxidant enzyme NAD(P)H: Quinone Oxidoreductase (NQO) in cultured astroglial cells. *Life Sciences* **66**, 2277-2288

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Myhrman R (2002) Detection and removal of L-Dopa in the legume *Mucuna*. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 142-163. Available at Online: www.cidicco.hn

Nachama SI, Edward RK (1967) Does 3,4-dihydroxyphenylalanine play a part in favism? *Nature* **215**, 285-286

Nogueira MA, de Oliveira JS, Ferraz S (1996) Nematicidal hydrocarbons from *Mucuna aterrima*. *Phytochemistry* **42**, 997-998

Nyirenda D, Musukwa M, Jonsson LO (2003) The effects of different processing methods of velvet beans (*Mucuna pruriens*) on L-Dopa content, proximate composition and broiler chicken performance. *Tropical Subtropical Agroecosystems* **1**, 253-260

Pardo B, Mena MA, Casarejos MJ, Paino CL, Yebenes JGD (1995) Toxic effects of L-Dopa on mesencephalic cell cultures prediction with antioxidants. *Brain Research* **682**, 133-143

Park HS, Lee JY, Kim HS (1998) Production of L-Dopa (L-3,4-Dihydroxyphenylalanine) from benzene by using a hybrid pathway. *Biotechnology and Bioengineering* **58**, 339-343

Pavlis M, Feretti C, Levy A, Gupta N, Linster C (2006) L-Dopa improves odor discrimination learning in rats. *Physiology and Behavior* **87**, 109-113

Pérez-Hernández F, Ayala-Burgos AJ, Belmar-Casso R (2003) Performance of growing lambs supplemented with *Mucuna pruriens*. *Tropical Subtropical Agroecosystems* **1**, 119-122

Perez-Ruiz T, Martinez-Lozone C, Tomas V, Val O (1993) Application of photochemical inhibition in flow injection systems: Determination of epinephrine and L-Dopa. *Talanta* **40**, 1625-1630

Pfutzner W, Przybilla B (1997) Malignant melanoma and levodopa: Is there a relationship? Two new cases and a review of the literature. *Journal of the American Academy of Dermatology* **37**, 332-336

Pialis P, Saville BA (1998) Production of L-Dopa from tyrosinase immobilized on nylon 6,6: enzyme stability and scale up. *Enzyme and Microbial Technology* **22**, 261-268

Prakash D, Tewari SK (1999) Variation on L-Dopa contents in *Mucuna* species. *Journal of Medicinal and Aromatic Plant Science* **21**, 343-346

Pras N, Woerdenbag HJ, Batterman S, Visser JF, Van Uden W (1993) *Mucuna pruriens*: improvement of the biotechnological production of the anti-Parkinsonian drug L-Dopa by plant cell selection. *Pharmacy World and Science* **15**, 263-268

Price M (2002) The L-Dopa problem as viewed by community development workers. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 111-118. Available online: www.cidicco.hn

Pugalenthi M, Vadivel V (2006) Agrobiodiversity of eleven accession of *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Baker ex Burck (velvet bean) collected from four districts of South India. *Genetic Resources and Crop Evolution* **54**, 1117-1124

Pugalenthi M, Vadivel V, Gurumoorthi P, Janardhanan K (2004) Comparative nutritional evaluation of little known legumes, *Tamarindus indica, Erythrina indica* and *Sesbania bispinosa*. *Tropical and Subtropical Agroecosystems* **4**, 107-123

Pugalenthi M, Vadivel V, Siddhuraju P (2005) Alternative food/feed perspectives of an under-utilized legume *Mucuna pruriens* var. *utilis*-a review. *Plant Foods for Human Nutrition* **60**, 201-218

Pugalenthi M, Vadivel V, Siddhuraju P, Gurumoorthi P, Janardhanan K (2003) Nutritional potential of an under-exploited legume, *Mucuna monosperma* DC ex Wight. *Indian Journal of Agricultural Biochemistry* **16**, 73-78

Qi AM, Ellis RH, Keatinge JDH, Wheeler TR, Tarawali SA, Summerfield RJ (1999) Differences in the effects of temperature and photoperiod on progress to flowering among diverse *Mucuna* species. *Journal of Agronomic and Crop Science* **182**, 249-258

Queneherve P, Topart P, Martiny B (1998) *Mucuna pruriens* and other rotational crops for control of *Meloidogyne incognita* and *Rotylenchulus reniformis* in vegetables in polytunnels in Martinique. *Nematropica* **28**, 19-30

Rabey JM, Shabtai H, Graff E, Harsat A, Korczyn AD (1993) Broad bean (*Vicia faba*) consumption and Parkinson's disease. *Advances in Neurology* **60**, 681-684

Rajaram N, Janardhanan K (1991) The biochemical composition and nutritional potential of the tribal pulse, *Mucuna gigantean* (Willd) DC. *Plant Foods for Human nutrition* **41**, 45-51

Randhir R, Shetty K (2007) Mung beans processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcer management. *Innovative Food Science and Emerging Technologies* **8**, 197-204

Randhir R, Shetty P, Shetty K (2002) L-Dopa and total phenolic stimulation in dark germinated fava bean in response to peptide and phytochemical elicitors.

Process Biochemistry **37**, 1247-1256

Ranjith NK, Sasikala C, Ramana CV (2007) Catabolism of L-Phenylalanine and L-Tyrosine by *Rhodobacter sphaeroides* OU5 occurs through 3,4-Dihydroxyphenylalanine. *Research in Microbiology* **158**, 506-511

Reddy KC, Soffes AR, Prine GM, Dunn RA (1986) Tropical legumes for green manure. II. Nematode populations and their effects on succeeding crop yields. *Agronomic Journal* **78**, 5-10

Rehr SS, Janzen DH, Feeny PP (1973) L-Dopa in legume seeds: A chemical barrier to insect attack. *Science* **181**, 81-82

Reynolds JEF (1989) *The Extra Pharmacopoeia*, The Pharmaceutical Press, London, UK, pp 1015-1020

Rodgers KJ, Hume PM, Dunlop RA, Deam RT (2004) Biosynthesis and turnover of L-Dopa containing proteins by human cells. *Free Radical Biology and Medicine* **37**, 1756-1764

Rodriguez-Kabana R, Pinochet J, Robertson DG, Wells L (1992) Crop rotation studies with the velvetbean (*Mucuna deeringiana*) for the management of *Meloidogyne* spp. *Journal of Nematology* **24**, 662-668

Roe DA (1985) Nutritional effects of anti-Parkinson drugs: L-Dopa. In: Roe DA (Ed) *Drug-Induced Nutritional Deficiencies*, The AVI Publishing Co., Inc., Connecticut, USA, pp 293-299

Rohn S, Rawel HM, Pietruschiski N, Kroll J (2001) *In vitro* inhibition of a-chymotryptic activity by phenolic compounds. *Journal of the Science of Food and Agriculture* **81**, 1512-1521

Roozen PJ, de Groot (1985) Electrophoresis and assay of trypsin inhibitors in different stages of *tempe* production. *Journal of Food Biochemistry* **9**, 37-48

Roy DN (1981) Toxic amino acids and proteins from *Lathyrus* plants and other leguminous species: A literature review. *Nutrition Abstract Reviews* **51**, 691-707

Sahid I, Tasrif A, Sasroutomo SS, Latiff A (1993) Allelopathic potential of legume cover crops on selected weed species. *Plant Protection Quarterly* **8**, 49-53

Sanginga N, Ibewiro B, Houngnandan P, Vanlauwe B, Okogun JA, Akobundu IO, Versteeg M (1996) Evaluation of symbiotic properties and nitrogen contribution of *Mucuna* to maize grown in the derived savanna of West Africa. *Plant and Soil* **179**, 119-129

Sayyed IA, Sudalai A (2004) Asymmetric synthesis of L-Dopa and (*R*)-seleginine via OsO$_4$-catalyzed asymmetric dihydroxylation. *Tetrahedron Asymmetry* **15**, 3111-3116

Schinelli S, Paolillo M, Quartieri M, Santagostino G (1993) Dopamine synthesis, uptake and metabolism in embryonic rat mesencephalic cultures. *Pharmacological Research* **28**, 265-269

Shen J, Zhao S (2004) Enantiomeric separation of naphthalene-2,3-dicarboxaldehyde derivatized D,L-3,4-Dihydroxyphenylalanine and optical purity analysis of L-3,4-Dihydroxyphenylalanine drug by cyclodextrin-modified micellar electrokinetic chromatography. *Journal of Chromatography A* **1059**, 209-214

Shetty P, Atallah MT, Shetty K (2002) Effect of UV treatment on the proline-linked pentose phosphate pathway for phenolics and L-Dopa synthesis in dark germinated *Vicia faba*. *Process Biochemistry* **37**, 1285-1295

Shi Y, Benzie IFF, Buswell JA (2002) L-Dopa oxidation products prevent H$_2$O$_2$-induced oxidative damage to cellular DNA. *Life Sciences* **71**, 3047-3057

Siddhuraju P (2006) The antioxidant activity and free radical-scavenging capacity of phenolics of raw and dry heated moth bean (*Vigna aconitifolia* Jacq. Marechal) seed extracts. *Food Chemistry* **99**, 149-157

Siddhuraju P (2007) Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. *LWT Food Science and Technology* **40**, 982-990

Siddhuraju P, Becker K (2001a) Effect of various domestic processing methods on antinutrients and *in vitro* protein and starch digestibility of two indigenous varieties of Indian tribal pulse, *Mucuna pruriens* var. *utilis*. *Journal of Agricultural and Food Chemistry* **49**, 3058-3067

Siddhuraju P, Becker K (2001b) Rapid reverse-phase high performance liquid chromatographic method for the quantification of L-Dopa (L-3,4-dihydroxyphenylalanine), non-methylated and methylated tetrahydroisoquinoline compounds from *Mucuna* beans. *Food Chemistry* **72**, 389-394

Siddhuraju P, Becker K (2001c) Preliminary nutritional evaluation of *Mucuna* seed meal (*Mucuna pruriens* var. *utilis*) in common carp (*Cyprinus carpio* L.): An assessment by growth performance and feed utilization. *Aquaculture* **196**, 105-123

Siddhuraju P, Becker K (2002) Effect of phenolic nonprotein amino acid L-Dopa (L-3,4-dihydroxyphenylalanine) on growth performance, metabolic rates and feed nutrient utilization of common carp (*Cyprinus carpio* L.). *Aquaculture Nutrition* **8**, 69-77

Siddhuraju P, Becker K (2003a) Comparative nutritional evaluation of differentially processed mucuna seeds (*Mucuna pruriens* (L.) DC. Var. *utilis* (Wall ex Wight) Baker ex Burck) on growth performance, feed utilization and body composition in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture Research* **34**, 487-500

Siddhuraju P, Becker K (2003b) Studies on antioxidant activities of *Mucuna* seed (*Mucuna pruriens* var. *utilis*) extract and various non-protein amino/imino acids through *in vitro* models. *Journal of Science of Food and Agriculture* **83**, 1517-1524

Siddhuraju P, Becker K (2005) Nutritional and antinutritional composition, *in vitro* amino acid availability, starch digestibility and predicted glycemic index of differentially processed mucuna beans (*Mucuna pruriens* var. *utilis*): an under-utilized legume. *Food Chemistry* **91**, 275-286

Siddhuraju P, Becker K (2007) The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chemistry* **101**, 10-19

Siddhuraju P, Becker K, Makker HPS (2000) Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilized tropical legume, *Mucuna pruriens* var. *utilis*. *Journal of Agricultural and Food Chemistry* **48**, 6048- 6060

Siddhuraju P, Manian S (2007) The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food Chemistry* **105**, 950-958

Siddhuraju P, Vijayakumari K, Janardhanan K (1996) Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.) *Journal of Agricultural and Food Chemistry* **44**, 2636-2641

Spencer JPE, Jenner A, Aruoma OI, Evans PJ, Kaur H, Dexter DT, Jenner P, Lees AJ, Marsden DC, Halliwell B (1994) Intense oxidative DNA damage promoted by L-Dopa and its metabolites, implications for neurodegenerative disease. *FEBS Letters* **353**, 246-250

Spencer JPE, Jenner A, Butler J, Aruoma OI, Dexter DT, Jenner P, Halliwell B (1996) Evaluation of the pro-oxidant and antioxidant actions of L-Dopa and dopamine *in vitro*: Implications for Parkinson's disease. *Free Radical Research* **24**, 95-105

Spina MB, Cohen G (1988) Exposure of school synaptosomes to L-Dopa increases levels of oxidized glutathione. *Journal of Pharmacology Experimental Therapy* **247**, 502-507

Spratto GR, Woods AL (1996) *Delmar's NDR-96: Nurse's Drug Reference*, Delmar Publishers, Boston, pp 779-782

Srivastava S, Khokhar S (1996) Effects of processing on the reduction of β-ODAP (β-N-oxylyl-L-2,3-diaminopropionic acid) and antinutrients of khesari dhal, *Lathyrus sativus*. *Journal of Agricultural and Food Chemistry* **71**, 50-58

Standaert DG, Young AB (1996) Treatment of central nervous system degenerative disorders. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG (Eds) *The Pharmacological Basis of Therapeutics*. 9 Ed.. McGraw Hill, New York, pp 503-519

Szabo NJ, Tebbett IR (2002) The chemistry and toxicity of *Mucuna* species. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 120-141. Available online: www.cidicco.hn

Szczypka MS, Zhou GY, Palmiter RD (1998) Dopamine stimulated sexual behaviour in testosterone dependent mice. *Behavioral Neuroscience* **112**, 1229-1235

Szent-Kiralyi I (1979) Analytical methods for the determination of L-Dopa in seeds and fresh and dry plant materials. *Planta Medica* **36**, 291-292

Takasaki S, Kawakishi S (1997) Formation of protein bound 3,4 dihydroxyphenylalanine and 5-S-cysteinyl-3,4-dihydroxyphenylalanine as new cross linkers in gluten. *Journal of Agricultural and Food Chemistry* **45**, 3472-3475

Teixeira AA, Rich EC, Szabo NJ (2003) Water extraction of L-Dopa from *Mucuna* bean. *Tropical Subtropical Agroecosystems* **1**, 159-171

Teixeira MFS, Bergamini MF, Marques CMP, Bocchi N (2004) Voltammetric determination of L-Dopa using an electrode modified with trinuclear ruthenium amine complex (Ru red) support on Y0type zeolite. *Talanta* **63**, 1083-1088

Teixeira MFS, Marcolino-Junior LH, Fatibello-Fitho O, Doacka ER, Bergamini MF (2007) An electrochemical sensor for L-Dopa based on Oxovanadium-salen thin film electrode applied flow injection system. *Sensors and Actuators B Chemical* **122**, 549-555

Temple SR, Huyck LM (2002) Genetic improvement of *Mucuna* to reduce antinutritional factors and its ecological significance. In: Flores M, Eilitta M, Myhrman R, Carew LB, Carsky RJ (Eds) *Proceedings of an International Workshop "Food and Feed from Mucuna: Current Uses and the Way Forward"*, Tegucigalpa, Honduras, 26-29 April, 2000, pp 306-338. Available online: www.cidicco.hn

Thurston HD (1997) *Slash/Mulch Systems: Sustainable Methods for Tropical Agriculture*, Westview Press, Boulder, CO, pp 197-214

Topps JH, Oliver J (1993) Animal foods of central Africa. Technical handbook. *Zimbabwe Agricultural Journal* **154**, 164-167

Tripathi YB, Upadhyay AK (2001) Antioxidant property of *Mucuna pruriens* Linn. *Current Science* **80**, 1377-1378

Tsuda T, Ohshima K, Kawakishi S, Osawa T (1994) Antioxidant pigments isolated from the seeds of *Phaseolus vulgaris* L. *Journal of Agricultural and Food Chemistry* **42**, 248-251

Udensi UE, Akobundu IO, Ayeni AO, Chikoye D (1999) Management of cogongrass (*Imperata cylindrica*) with velvetbean (*Mucuna pruriens* var. *utilis*) and herbicides. *Weed Technology* **13**, 201-208

Ukachukwu SN, Obioha FC (1997) Chemical evaluation of *Mucuna cochinchinensis* as alternative protein feedstuff. *Journal of Applied Chemistry and Agricultural Research* **4**, 33-38

Ukachukwu SN, Szabo NJ (2003) Effect of processing, additives and vitamin b6 supplementation of *Mucuna pruriens* var *cochinchinensis* on broilers. *Tropical Subtropical Agroecosystems* **1**, 227-238

Vadivel V, Janardhanan K (1997) Agrobotanical characters of different germplasm of velvet bean (*Mucuna pruriens* var. *utilis* Wall ex. Wight). *Journal of Swamy Botanical Club* **14**, 37-40

Vadivel V, Janardhanan K (2001) Nutritional and antinutritional composition of velvet bean: An under-utilized food legume in South India. *International Journal of Food Science and Nutrition* **51**, 279-287

Vadivel V, Pugalenthi M (2006) Analysis of toxic/antinutritional constituents and *in vitro* protein digestibility of eleven accessions of velvet bean: An under-utilized food legume. *The Indian Journal of Nutrition and Dietetics* **43**, 350-356

Vadivel V, Pugalenthi M (2007) Biological value and protein quality of raw and processed seeds of *Mucuna pruriens* var. *utilis*. *Livestock Research for Rural Development* **19**, 97-99

Valdes RH, Puzer L, Gomes M, Marques ESJ, Aranda AG, Bastos ML, Gemal AL, Antunes OAC (2004) Production of L-Dopa under heterogeneous asymmetric catalysis. *Catalysis Communications* **5**, 631-634

Valverde MT, Alster FA, Zakrzewska EI, McMurtry JP, Vasilatos-Younken R, Gernat AG and Carew LB (2000) Organ growth and blood chemistry in growing chickens fed L-Dopa. *World's Poultry Science Journal* **56**, 548-551

Versteeg MN, Amadji F, Eteka A, Koudokpon V, Manyong VM (1998) Collaboration increase in the use of *Mucuna* in production systems in Benin. In: Buckles D, Eteka A, Osiname O, Galiba M, Galiano G (Eds) *Cover Crops in West Africa Contributing to Sustainable Agriculture*, IDRC, Ottawa, Canada; IITA, Ibadan, Nigeria; Sasakawa Global 2000, Cotonou, Benin, pp 33-43

Vijayakumari K, Siddhuraju P, Janardhanan K (1996) Effect of different post-harvest treatments on antinutritional factors in seeds of the tribal pulse, *Mucuna pruriens* (L.) DC. *International Journal of Food Science and Nutrition* **47**, 263-272

Vijayakumari K, Smitha KB, Janardhanan K (2002) Biochemical characterization of the tribal pulse, *Mucuna utilis* Wall ex. Wight. Seeds. *Journal of Food Science and Technology* **39**, 650-653

Wanjekeche E, Wakasa V, Mureithi JG (2003) Effect of germination, alkaline and acid soaking and boiling on the nutritional value of mature and immature *Mucuna* (*Mucuna pruriens*) beans. *Tropical Subtropical Agroecosystems* **1**, 183-192

Wichers HJ, Malingré TM, Huizing HJ (1983) The effect of some environmental factors on the production of L-Dopa by alginate-entrapped cells of *Mucuna pruriens*. *Planta* **158**, 482-486

Wichers HJ, Pras N, Huizing HJ (1989) *Mucuna pruriens: In vitro* production of L-Dopa. In: Bajaj YPS (Ed) *Biotechnology in Agriculture and Forestry (Vol 7) Medicinal and Aromatic Plants II*, Springer-Verlag, Berlin, Germany, pp 457-463

Yen GC, Duh PD (1993) Antioxidant properties of methanolic extracts from peanut hulls. *Journal of American Oil Chemical Society* **70**, 383-386

Zenin CT, Park VK (1978) Isoenzymes of polyphenol oxidase from high L-Dopa containing velvet bean. *Journal of Food Science* **43**, 646-647

Zhao P, Li J, Wang Y, Jiang H (2007) Broad-spectrum antimicrobial activity of the reactive compounds generated *in vitro* by *Manduca Sexta* phenoloxidase. *Insect Biochemistry and Molecular Biology* **37**, 952-959