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Bioactive compounds in velvet bean seeds: A promising high quality legume to attain food security in developing countries

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Introduction

Apart from common legume seeds, the earlier research efforts revealed the nutritive potential of certain promising under-utilized/wild legume seeds, including the pulses of tribal utility (JANARDHANAN ET AL., 2003). Among the various under-utilized legumes, the *Mucuna pruriens* (velvet bean, VB) seeds merit a wider use as a food legume in many tropical and sub-tropical countries (PUGALENTHI ET AL., 2010). It is widespread in Southern and South-eastern Asian regions and also cultivated as a green manure/cover crop in some parts of Africa and South America. In India, the mature VB seeds are being traditionally consumed by certain ethnic groups, particularly the Kanikkar, Lambadi, Uraali and Dravidian tribes living in Tamilnadu, Kerala, Karnataka and Andhrapradesh States. Similarly, the seed materials of VB are being eaten by the native people in different parts of the world like Benin, Brazil, Ghana, Guatemala, Guinea, Kenya, Malawi, Mexico, Nigeria, Philippines, Sri Lanka and Zimbabwe. Few reports indicating that the VB seeds possess appreciable levels of certain bioactive compounds such as total free phenolics, tannins, phytic acid and L-Dopa (3,4-Dihydroxyphenylalanine, a non-protein phenolic amino acid), in addition to high protein content (26 – 29%) and other nutrients (VADIVEL AND PUGALENTHI, 2010). Hence, in recent years, research efforts are under-way for the utilization of VB seeds in the dietary management of Parkinsonism, diabetes, obesity *etc*.

Generally before consumption, all the legume grains are subjected to appropriate processing methods in order to improve their nutritive quality. But, most of the common processing methods have been shown to reduce the levels of bioactive compounds in commercial legume grains. In VB seeds also, a significant level of loss of bioactive compounds was reported during soaking, cooking and autoclaving treatments (PUGALENTHI ET AL., 2010). So, it is very important to identify a suitable processing method, which will cause minimum loss of bioactive compounds in VB seeds. In this connection, in the present study, an attempt has been made to analyze the effect of certain indigenous processing techniques particularly those are used by Indian tribal groups, on the levels of bioactive compounds of VB seeds collected from different parts of the world.

Materials and Methods

Seed samples

The details on collection of seed materials of VB from different agro-climatic locations of the world were given in Table 1.

Processing methods

Soaking in tamarind solution and cooking**:** The tamarind solution was prepared by dissolving 50 g of tamarind pulp in 500 ml of distilled water (pH 2.75). The whole seeds of VB (50 g) were soaked in tamarind solution in the ratio of 1:10 (w/v) and kept in dark for 8 h at 25°C. The soaked samples were rinsed and then cooked with distilled water at 85-90°C on a hot plate until they became soft when felt between the fingers (about 45 min).

Soaking in alkaline solution and cooking**:** The whole seeds of VB (50 g) were soaked in 500 ml of 0.2% $NaHCO_3$ solution (pH 8.6) for 8 h and then cooked as described above.

Sprouting and oil-frying**:** VB seeds (50 g) was added into the red-soil suspension and mixed well. The tray was covered with a moist cloth and kept for 7 days in dark at 25°C. Then the sprouts were separated and thoroughly washed with tap water. The sprouts, thus obtained, were fried with Biskin oil (100% sunflower oil) at 85-90°C on a hot plate for about 15 min.

Open-pan roasting**:** The VB seeds (50 g) were taken in an iron pot and roasted for 30 min at 120-130°C.

Analysis of bioactive compounds

All the processed as well as raw samples were freezed at -80°C and freeze-dried for 48 h. Then the samples were first cracked with the help of a wooden hammer into small pieces and subsequently powdered in a seed mill (Siemens, Germany) to 1 mm particle size, freeze-dried for 24 h and stored at 9°C until further use. The total free phenolics were extracted and estimated according to the method of SINGLETON ET AL. (1999) where as the tannins were quantified by using vanillin reagent method (PRICE ET AL., 1978). The L-Dopa content was determined by measuring the ultra-violet light absorption at 282 nm according to BRAIN (1976). The phytic acid level was determined according to LATTA AND ESKIN method (1980).

Statistical analysis

All the data were analyzed and expressed as means ± standard deviation of five separate determinations (n = 5). The statistical analysis was carried out by using SPSS for Windows (SPSS Inc., Chicago, IL, version 11.0).

Results and Discussion

Total free phenolics

The total free phenolics content of raw VB accessions collected from various parts of the world were found to range between 5.24 and 8.65 g/100 g seed flour DM (Figure 1). It is interesting to notice that the VB accessions with black coloured seed coat (VB accessions 3, 8 and 10) registered significantly ($p < 0.05$) higher level of total free phenolics when compared to white coloured and mottled seed coat VB accessions. In addition to seed coat colour, it is well documented that the quantity of phenolic compounds in seed samples is influenced by soil, environmental conditions, genotype (cultivar/variety), agronomic practices (irrigation, fertilization and pest management), maturity level at harvest and post-harvest storage. Since VB grows wildly in adverse environmental conditions such as drought, poor soil *etc*., a high phenolic content contributes to the resistant function.

Tannins

The tannins content of raw VB seeds were found to falls between 1.77 and 3.49 g/100 g DM (Figure 1). It is noticeable that, the black colour seed coated VB accessions collected from Zimbabwe, Mexico and Guinea registered significantly ($p < 0.05$) higher level of tannins. It is postulated that high level of condensed tannins or proanthocyanidin are seen in coloured beans than in yellow or white coloured beans. Since, the level of phenolics was relatively low in pale coloured seeds; it is possible to assume that the major phenolics in dark coloured coated seeds could be proanthocyanidins. Recent studies have demonstrated a quantitative pattern of heredity for tannins content and that tannins level is also associated with seed coat colour inheritance. Several factors, such as plant type, cultivar, age of the plant or plant parts, stage of development and environmental conditions were reported to govern the tannins content in legume grains. Presence of high content of tannins in VB seeds might be due to the metabolism of polyphenolic compounds or polymerization of existing phenolic compounds during development or maturation.

L-Dopa

L-Dopa (L-3,4-Dihxdroxyphenylalanine) is a non-protein phenolic amino acid, mainly used in the treatment of Parkinson’s disease, since it is the precursor of dopamine. L-Dopa has also been investigated as a dietary supplement to manage hypertension, renal failure and liver cirrhosis. Further, the protective effects of L-Dopa on small bowel injury, ulcer, gastro-intestinal diseases, diabetes as well as antioxidant stress were scientifically proved by earlier studies (Pugalenthi et al., 2005). The raw seed materials of different VB accessions of the present study showed the L-Dopa content of 4.30 - 6.23 g/100 g DM, while the Guatemala accession recorded the maximal level (Figure 1). Such a wide variability in L-Dopa content among VB accessions was reported to cause by both environmental effect and genetic nature. Presence of more L-Dopa was noticed in the VB plants growing near the equator (within 10°) than the plants cultivated far away from equatorial regions in earlier investigations (PUGALENTHI ET AL., 2003). Further, the L-Dopa synthesis is reported to be high in plants growing at low latitudes, near the equator. It was also hypothesized that variation in the intensity of light and backscattered ultraviolet radiation, both generally more near the equator, may be among the factors explaining why the L-Dopa content was found to be high in VB plants growing at low latitudes.

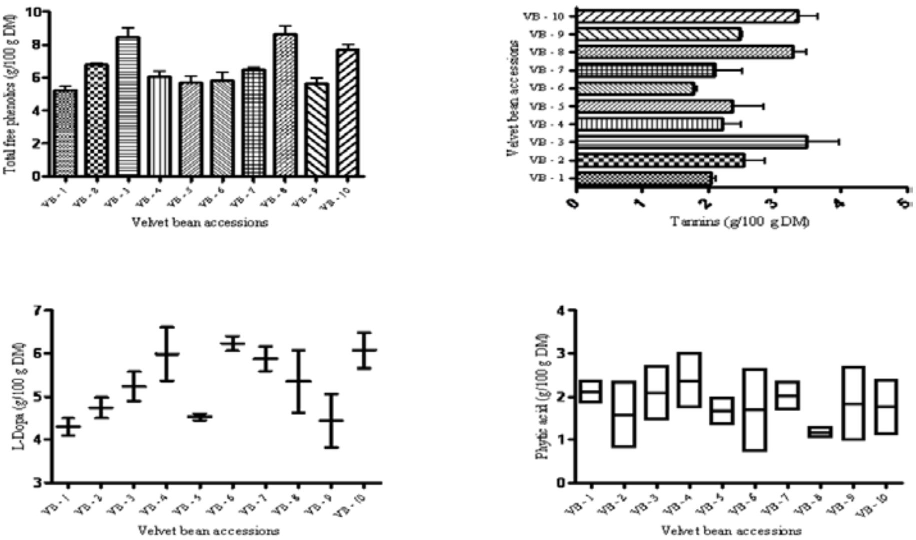

Figure 1 Level of bioactive compounds in ten different accessions of velvet bean seeds
A: Total free phenolics, B: Tannins, C: L-Dopa and D: Phytic acid

Phytic acid

In recent years, the phytic acid is considered as an antioxidant, anti-carcinogenic, hypoglycemic and hypolipidemic agent, in addition to the fact that a high phytate diet can be effectively used in the treatment of hyper-calciuria and kidney stones in human beings. The raw VB seeds of different accessions were found to contain 1.17 - 2.37 g/100 g DM of phytic acid (Figure 1). Further, such variability might be attributed to both genetic and environmental conditions. In general, the cultivar, which contains appreciably high amount of protein is observed to be associated with high phytic acid content. Hence, as the protein content increases, the phytate level is also found to increase in the seed samples. The amount of phytic acid is always exceeds than that of phosphorus for all the legume cultivars, which indicates that the ratio would be more than 100%. Factors that affect the total phosphorous content, such as soil, available phosphorous and fertilizer can also influence the phytic acid concentration.

Effect of processing methods

Soaking in tamarind solution and cooking: Reduction of significant level ($p < 0.05$) of total free phenolics (24 – 46%), tannins (16 – 57%), L-Dopa (24 – 44%) and phytate (25 – 47%) (data not shown) were noticed in most of the VB accessions during soaking in tamarind solution + cooking treatment. Soaking in tamarind solution may leads to softening of cell wall tissues under acidic environment, which is usually accompanied by release of bounded phytochemical compounds, and hence may be leached into the soaking medium. Degradation of heat sensitive compounds under cooking at high temperature is also attributed such a loss of bioactive compounds in VB seeds.

Soaking in alkaline solution and cooking: Soaking in alkaline solution + cooking treatment resulted in significant loss of total free phenolics (36 – 65%), tannins (24 – 52%), L-Dopa (48 – 67%) and phytate (18 – 37%) (data not shown). Such significant level of reduction of phytochemical compounds during this treatment might be because of the leaching out of compounds into the soaking medium due to increased permeability of the seed coat under alkaline environment or due to solubilisation of this compound in alkaline solution under the influence of concentration gradient and also due to degradation of compounds with the high temperature during subsequent cooking.

Sprouting and oil-frying: Total free phenolics (4 – 11%) and tannins (1 – 8%) were slightly increased, but drastic loss of L-Dopa (48 – 67%) and non-significant level of removal of phytic acid (11 – 27%) were noticed during sprouting + oil-frying (data not shown). Earlier research studies indicated that, a major portion of total free phenolics was stored in seeds as soluble conjugate or insoluble forms. Hence, the little level of increase noticed in phenolics and tannins content of VB seeds under sprouting + oil-frying treatment might be due to mobilization of bound phenolics by the activation of polyphenol oxidase enzyme during sprouting and also due to release of free phenolics from bounded form by the breakdown of cellular constituents and cell walls during subsequent thermal process (oil-frying). Further, dissociation of conjugated phenolic forms due to oil-frying followed by some polymerization/oxidation of phenolic constituents may also be responsible for the increase.

Open-pan roasting: Significant level of reduction of total free phenolics (24 – 44%) and tannins (31 – 45%) and moderate reduction of L-Dopa (6 – 17%) and phytic acid (5 – 14%) were noticed during open-pan roasting (data not shown). Degradation of phytochemical compounds as a result of direct heat exposure could be a reason for this reduction observed in VB seeds under open-pan roasting.

Conclusions and Outlook

Among the VB accessions, significantly high levels of total free phenolics and tannins were noticed in VB seeds collected from Zimbabwe, Mexico and Guinea while Guatemala VB accession registered maximum level of L-Dopa. Considering the effect of different indigenous processing methods, soaking in tamarind solution + cooking as well as soaking in alkaline solution + cooking treatments represented more aggressive practices and exhibited drastic level of loss of all the investigated compounds. Further, open-pan roasting also demonstrated a significant level of reduction of total free phenolics, tannins with moderate loss of L-Dopa and phytic acid. Nonetheless, sprouting + oil-frying was found to slightly increase the content of total free phenolics and tannins and also caused only minimal loss of L-Dopa and phytic acid. Hence, such viable and mild processing method could be recommended for the consumption of VB seeds in order to increase the dietary intake of health beneficial bioactive compounds.

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