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PROCESSING OF VELVET BEAN (*Mucuna pruriens* var *utilis*) BY FERMENTATION

M. Egounlety

Département de Nutrition & Sciences Alimentaires,

Faculté des Sciences Agronomiques, Université d'Abomey-Calavi,

Abomey-Calavi, Bénin 01 BP 526 (FSA/UAC)

E-mail: egoumout@syfed.bj.refer.org

SUMMARY

Velvet bean or *Mucuna* (*Mucuna pruriens* var *utilis*) was pre-treated and subjected to fungal or bacterial fermentation to produce three foods, namely *Mucuna* tempe, *Mucuna* condiment and *Mucuna*-fortified weaning foods. pH, total crude protein, soluble constituents as well as the residual levels of the toxic principle, L-Dopa, were evaluated.

pH increased in mould and basic fermentations while it decreased in lactic acid fermentation. Total crude protein remained fairly constant in all types of fermentations. Levels of soluble solids and soluble protein increased dramatically in fungal and basic fermentations. Non-protein nitrogen followed the same trend.

Pre-treatment of the bean before fermentation provided the most effective means of reducing L-Dopa in velvet bean. In the treatment that reduced about 90% (89.95%) of L-Dopa, the beans were boiled for 45 min, dehulled, soaked for 12 or 24 hr with removal and replacement of water after 12 hr, then boiled in fresh water for an additional 45 min. Further reduction was obtained during fungal fermentation, suggesting the production of an L-Dopa-degrading enzyme. All the fermented products contained low levels of L-Dopa (often <0.1% dry wt). Production of *Mucuna* tempe and *Mucuna*-fortified weaning foods is recommended at household level as a way to alleviate protein-energy malnutrition. However, large-scale production is limited by the dehulling process.

Key words: velvet bean, *Mucuna*, fermentation, tempe, L-Dopa, weaning foods, condiment.

INTRODUCTION

Velvet bean (*Mucuna pruriens*) is an excellent cover crop and soil improver (Osei-Bonsu *et al*., 1993; Carsky *et al*., 1998). In addition, it commonly produces 200 to 600 kg of seeds per hectare which are very rich in protein (24-31%). However, the regular use of velvet bean for soil fertility enhancement is hampered by the lack of appropriate processing techniques of the seeds (Versteeg *et al*., 1998). Like many other grain legumes, velvet bean contains many anti-nutritional factors; unlike most grain legumes, it also contains L-Dopa, which has a number of anti-physiological effects. The adoption of velvet bean could be promoted through increased human or animal consumption of its seeds; therefore, fermentation techniques were tested as potential processing methods.

Fermentation is a widely practised, traditional, and a very economical method of food and beverage production in developing countries (Cooke *et al*., 1987; Sasson, 1988). Fermentation brings about numerous biochemical, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents, the reduction of anti-nutritional factors in grain legumes and the synthesis of B-vitamins (Djurtoft and Nielsen, 1984; Egounlety and Aworh, 1995; 2000; Barampama and Simards, 1994; Egounlety, 1994; 1996; 1998; Egounlety *et al*., 2002). Many fermented foods have been reported to be acceptable to West Africans (Egounlety and Syarief, 1992; Egounlety, 2001).

Three widely-consumed traditional fermented foods were selected for the processing of velvet bean: 1. a condiment, made of locust bean (*Parkia biglobosa*) both commercially and at household level in West and Central Africa through natural fermentation; 2. *tempe*, an oriental soybean (*Glycine max* Merr.) food which is fermented with *Rhizopus oligosporus* and is produced commercially in Indonesia and Malaysia; and 3. a cereal-based food used to wean children, also fermented naturally at household level throughout the whole Africa.

The objective of this study was to apply the fermentation techniques to velvet bean grains in order to reduce or eliminate the toxic principle.

MATERIALS AND METHODS

The materials

Velvet bean seeds (*Mucuna pruriens* var *utilis*) were provided by the International Institute of Tropical

Agriculture (IITA), Bénin Station, Cotonou, Bénin. Powder-form *tempe* inoculum (*Rhizopus oligosporus)* was provided by the National Nutrition Research and Development Centre, Bogor, Indonesia[1]. The viability of the inoculum was renewed on rice. Rice, maize and shelled seeds of melon (*Citrullus vulgaris*) were bought at Dantokpa market, Cotonou, Benin.

Preparation of velvet bean seeds

Different pre-treatments were used for the three types of foods. For velvet bean condiment, velvet bean seeds were boiled for 45 min in a 1:6 bean:water ratio (P/V=1/6), hand-dehulled, chopped into 2-3 pieces, soaked for 12 hr and reboiled for 45 min. For the velvet bean *tempe* and for the velvet bean weaning food, velvet bean seeds were boiled for 45 min (P/V=1/6), hand-dehulled, chopped into 2-3 pieces, soaked twice (P/V=1/3) for 12 hr with removal of soak water after each soaking period, recooked for 45 min (P/V=1/6) and drained. (Figure 1).

Production of velvet bean foods

Production of velvet bean condiment with natural bacteria involving Bacillus spp.

Drained boiled grains obtained from 1 kg of dry seeds were packed hot in calabash covered with polyethylene films to allow a natural bacterial (basic) fermentation (usually involving *Bacillus* spp.) similar to that of locust bean. The fermentation duration was 48 hr (Figure 1).

Production of velvet bean tempe with fungal inoculum (Rhizopus oligosporus)

Drained boiled grains obtained from 1 kg of dry seeds were cooled by spreading in a flat tray, inoculated with *tempe* starter (*R. oligosporus*; 0.4 g kg^{-1} dry bean), packed in polyethylene perforated bags (of 50 µm thickness), and fermented on racks at room temperature for 48 hr (Figure 1).

Production of weaning foods with maize-velvet bean (and melon) with natural lactic acid bacteria

Cooled boiled seeds obtained from 1.5 kg of dry seeds were milled and mixed with milled maize that has been soaked (48-72 hr) with or without the addition of cooked milled melon seeds. Upon the addition of water, the mixture was wet-sieved through 1mm mesh size (P/V=1/8) and co-fermented (simultaneous fermentation) at room temperature for 48 hr (Figure 1) (Egounlety, 2000). Co-fermentation involved either maize-velvet bean or maize-velvet bean-melon and was done with natural lactic acid bacteria. The ratios of ingredients were 50:50 and 50:40:10, respectively, for maize-velvet bean and maize-velvet bean-melon seed mixtures. Soaked maize itself was the source of the lactic acid bacteria and yeasts necessary for this fermentation (Wood and Hodge, 1985).

Determination of pH

pH is the appropriate parameter to follow when monitoring the fermentation process. Twenty grams of unfermented or fermented beans (natural basic and fungal fermentation) taken at 12 hr intervals were prepared into a homogenate (90-100 mL) by blending with 80 mL of distilled water for 2 min at maximum speed (Steinkraus *et al*., 1960). The pH was read on a Hanna Instruments 8418 pH-meter. In the case of the co-fermentation process, the pH was measured on the slurry also taken at 12 hr intervals. pH values of 7.0, 6.0 - 6.6 and 3.8 - 3.9 have been reported as the optimum for production of acceptable condiment, tempe and weaning foods, respectively (Egounlety, 1994; 2000).

Determination of total crude protein and soluble constituents during fermentation

Total crude protein was estimated by the Kjeldahl method (N × 6.25) on milled, freeze-dried, unfermented and fermented samples taken at 12 hr intervals.

Soluble solids, soluble protein (N × 6.25) and non-protein nitrogen were determined during fermentation. The above homogenate was rapidly heated to 80 °C in a water bath to stop further enzymatic activity, centrifuged at 3,000 rpm for 30 min and filtered through Whatman No.1 filter paper. The slurries resulting from maize-velvet bean mixtures were first centrifuged and filtered before heating to 80°C. Soluble solids and soluble nitrogen were determined by drying 5 mL of the filtrate at 70°C for 48 hr and by estimating the nitrogen content of it. In the case of non-nitrogen protein determination, 20 mL of filtrate was mixed with 20 mL of 10% trichloro-acetic acid solution, allowed to stand for 30 min, filtered and the nitrogen was estimated on 5 mL of this filtrate. Non-protein nitrogen is an indicator of protein hydrolysis up to the amino-acid step (Egounlety and Aworh, 2000).

Determination of L-Dopa

All samples were sent to Judson College, USA, for the determination of L-Dopa using the method of Myhrman

[1]The author attended a 3-month course on *tempe* technology in Indonesia in 1986 and brought the starter culture with him when he returned to Benin. He later conducted his Ph.D thesis on legume *tempe* processing.

(2002). Extraction with 5 mL of distilled water was performed on 0.1875 grams of flour ground to pass through a 40 mesh screen. The L–Dopa in the supernatant was analyzed by High Performance Liquid Chromatograghy using a Zorbax Stable Bond SB-C18 column (4.6 X 150 mm, 3.5 micron) with UV detection at 279 nm. The mobile phase was composed of two solutions; A made of 0.1 M phosphoric acid, 1 mM 1-octnesulfonic acid and 2 mM disodium EDTA and B, which was HPLC grade methanol. The solvents were mixed as a ratio of 90% A to 10% B at 1.0 mL min^{-1} for 15-20 min with an injection volume of 40 µl. L-Dopa (Sigma D-9628), 1.00 mM in water was used as the standard L-Dopa. The HPLC analysis was run in duplicate for each extraction solution. A total of 24 samples (a mixture of two replicate samples) were analyzed for L-Dopa content.

RESULTS AND DISCUSSION

Some observations during pretreatments and fermentation of velvet bean

During the pretreatment of the beans (Figure 1), the pH of the soaking water was 5.56 after the first soaking period and 4.46 after the second period. These conditions were favourable for the growth of the fungal microorganism or lactic acid bacteria involved in these fermentations. A dark water was obtained after the first soaking of cooked dehulled beans.

During fermentation of *Mucuna* with *R. oligosporus*, mycelium started growing at 24 hr and a strong firm white-brown sliceable cake was formed at 36 hr. A pleasant cheese-like smell was developed at that time and remained in the product up to 48 hr. In condiment fermentation, the colour became darker as fermentation proceeded. In the maize-velvet bean (and melon) fermentation, the colour of the slurry improved (becoming whiter) during fermentation of the mixture.

Biochemical changes during fermentation

Changes in moisture content, pH, water-soluble solids, total crude protein, water-soluble protein and non-protein nitrogen during fermentation of *Mucuna*-based foods are presented in Tables 1 to 4.

pH increased from 5.10 and 6.07 at the beginning to 5.98 and 7.08 at 48 hr during fermentation of *Mucuna* tempe and *Mucuna* condiment, respectively (Tables 1 and 2) while it decreased from 5.56 (maize-velvet bean) and 6.64 (maize-velvet bean-melon) to 3.24 and 4.42, respectively, in weaning products (Tables 3 and 4). Breakdown of sugars to lactic acid explains the decrease and low pH in weaning foods while protein hydrolysis dominates *Mucuna* tempe and condiment fermentations. Fungal and basic fermentation increased water-soluble solids, which rose from 4.65 and 3.12% at the beginning to 39.11 and 27.47% at 48 hr, respectively (Tables 1 and 2). This increase reflects a strong enzymatic action taking place during these fermentations.

Total crude protein remained fairly constant in all types of fermentations while soluble protein increased from 1.22 and 0.74% at the beginning to 19.42 and 16.98% at 48 hr in fungal and basic fermentation, respectively. In other words, these two processes solubilized 52.70 and 48.87% of the total protein. The same trend was observed for non-protein nitrogen, where 78.53 and 41.55% of total soluble nitrogen were in amino acid state in fungal and basic fermentations (Tables 1 and 2). This indicates that extensive breakdown of proteins occurred during these fermentation processes. In the natural lactic acid samples, soluble solids, although very low, decreased further and no soluble protein and non-protein nitrogen were detected during fermentation (Tables 3 and 4). The lactic acid bacteria are known to degrade sugars and need many growth factors.

The results recorded in this study on velvet bean *tempe* fermentation are in agreement with those of Steinkraus *et al*. (1960) and Paredes-Lopez *et al*. (1987). Increases in water-soluble solids from 13 to 28% and from 7.0 to 41.3% were reported in soybean and common bean (*Phaseolus vulgaris*), respectively, when fermented with *Rhizopus oligosporus* (Steinkraus *et al*., 1960; Paredes-Lopez *et al*., 1987). Moreover, Paredes-Lopez *et al*. (1987) noted that the fungal fermentation solubilized 41% of the total protein. *Rhizopus* sp. and *Bacillus* spp. involved in *tempe* and condiment fermentations produce protease that hydrolysed proteins to amino acids.

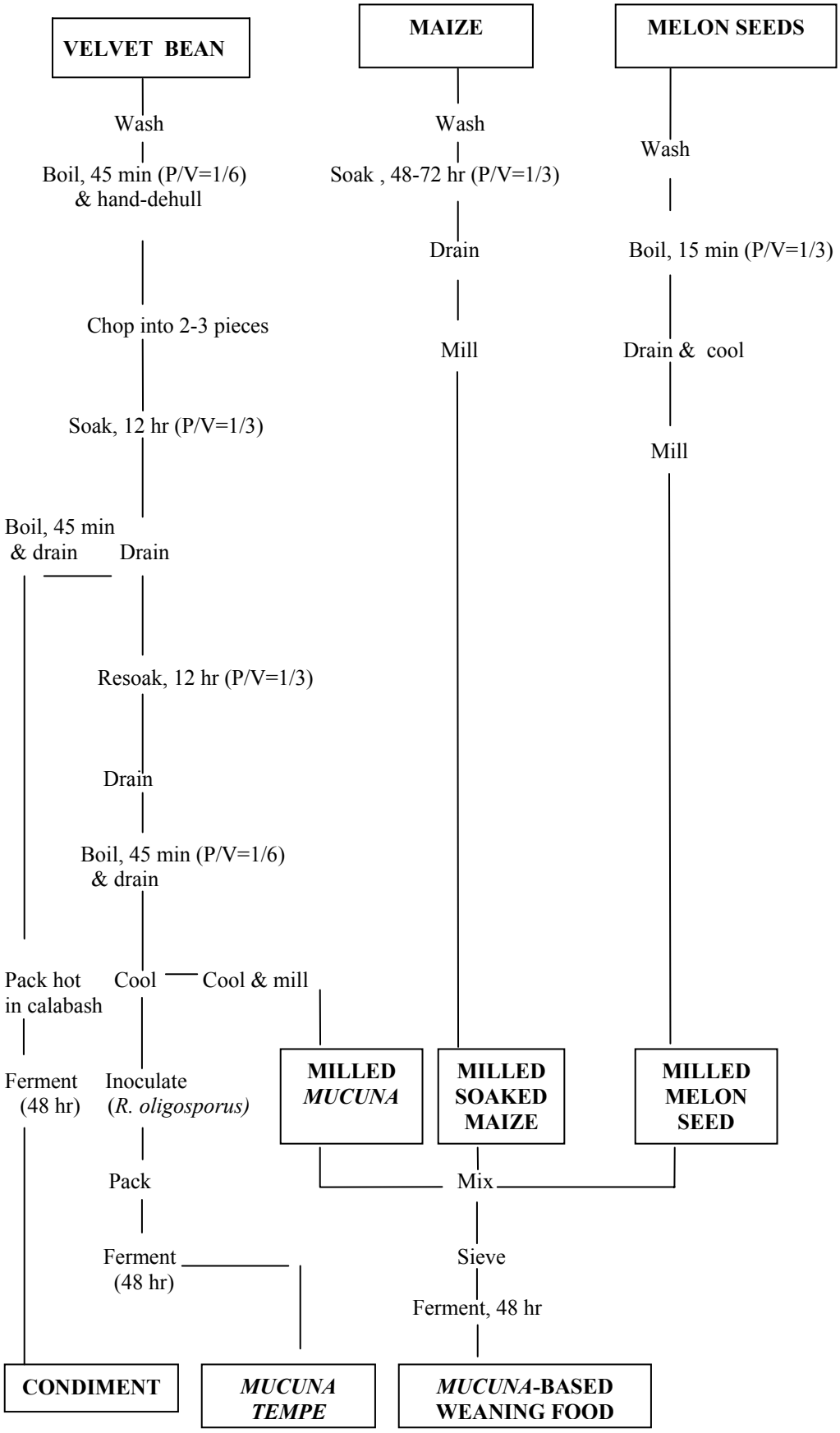

Figure 1. Flow diagram of production of fermented *Mucuna*-based foods.

Table 1. Effect of fermentation with *Rhizopus oligosporus* on moisture content, pH, soluble solids, total crude protein, soluble protein and non-protein nitrogen of velvet bean. Values presented are averages of two replicates.

Fermentation time (h)	Moisture content (%)	pH	Water-soluble solids	Total crude protein	Water-soluble protein	Non-protein nitrogen
			---------- (% DW) ----------			
0	56.54±1.97^{c}	5.10±0.01^{c}	4.65±0.05^{d}	30.48±0.06^{a}	1.22±0.09^{d}	0.14±0.01^{e}
12	56.21±2.23^{c}	5.14±0.01^{c}	4.84±0.09^{d}	30.62±0.05^{a}	1.55±0.08^{d}	0.75±0.03^{d}
24	58.48±2.16^{b}	5.36±0.02^{b}	24.81±0.05^{c}	32.25±0.16^{a}	7.18±0.58^{c}	1.56±0.04^{c}
36	59.59±1.04^{b}	5.84±0.03^{a}	32.22±0.15^{b}	35.75±0.04^{a}	9.38±0.15^{b}	2.45±0.07^{b}
48	63.03±0.45^{a}	5.98±0.03^{a}	39.11±0.16^{a}	36.85±0.16^{a}	19.42±0.67^{a}	4.63±0.15^{a}

DWB = Dry weight basis.
Means in a column followed by different letters are significantly different (P<0.05).

Table 2. Effect of fermentation with natural basic bacteria on moisture content, pH, soluble solids, total crude protein, soluble protein and non-protein nitrogen of velvet bean. Values presented are averages of two replicates.

Fermentation time (h)	Moisture content (%)	pH	Water-soluble solids	Total crude protein	Water-soluble protein	Non-protein nitrogen
			----------(% DW) ----------			
0	52.52±0.82^{c}	6.07±0.01^{c}	3.12±0.08^{d}	28.88±0.06^{a}	0.74±0.07^{d}	0.06±0.00^{d}
12	61.77±0.39^{a}	6.42±0.02^{b}	11.88±0.06^{c}	30.31±0.06^{a}	5.29±0.09^{c}	0.60±0.03^{c}
24	61.92±0.16^{a}	6.63±0.02^{b}	23.58±0.16^{b}	29.81±0.58^{a}	12.44±0.15^{b}	1.99±0.15^{b}
36	60.89±0.71^{b}	6.71±0.03^{b}	22.20±0.21^{b}	32.47±1.04^{a}	14.06±0.90^{b}	1.94±0.14^{b}
48	60.25±0.57^{b}	7.08±0.05^{a}	27.47±0.14^{a}	34.75±1.26^{a}	16.98±1.78^{a}	2.31±0.17^{a}

DWB = Dry weight basis.
Means in a column followed by different letters are significantly different (P<0.05).

Changes in L-Dopa

Data on the effect of the pre-treatments of the bean and fermentation of velvet bean foods on the levels of L-Dopa are presented in Table 5 and Figure 2. Raw *Mucuna* contained 6.36% of L-Dopa by the weight. Boiling for 45 min followed by dehulling reduced the original level by 25.89% to 4.71%. Soaking the cooked and dehulled bean for 12 hr reduced L-Dopa by 63.88% (to 2.30%) and a further 12 hr soaking after removal of soaking water reduced it by 78.63% (to 1.36%). 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 (Table 5).

The fermented products, especially the *Mucuna*-fortified weaning foods, contained low levels (often < 0.1%) of L-Dopa (Figure 2). However, the level of L-Dopa increased significantly during the first 12 hr of *Mucuna tempe* fermentation and the first 24 hr 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 1.197-1.203%, 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 (Figure 2).

Table 3. Effect of fermentation with natural lactic acid bacteria on moisture content, pH, soluble solids, total crude protein, soluble protein and non-protein nitrogen of mixture of velvet bean and maize (ratio 50 : 50). Values presented are averages of two replicates.

Fermentation time (h)	Moisture content (%)	pH	Water-soluble solids	Total crude protein	Water-soluble protein	Non-protein nitrogen
			(% DWB)			
0	73.98±1.04[c]	5.56±0.06[a]	5.45±0.05[a]	19.22±0.08[a]	0.50±0.01[a]	ND
12	72.15±0.19[d]	3.77±0.07[b]	2.37±0.07[b]	17.73±0.08[a]	ND	ND
24	74.81±1.67[b]	3.50±0.08[c]	2.07±0.16[b]	17.84±0.12[a]	ND	ND
36	75.37±0.98[b]	3.32±0.04[c]	2.11±0.33[b]	17.70±0.09[a]	ND	ND
48	77.79±0.20[a]	3.24±0.02[c]	1.89±0.09[b]	17.93±0.10[a]	ND	ND

DWB = Dry weight basis.
ND = Not detected.
Means in a column followed by different letters are significantly different (P<0.05).

Table 4. Effect of fermentation with natural lactic acid bacteria on moisture content, pH, soluble solids, total crude protein, soluble protein and non-protein nitrogen of mixture of maize, velvet bean and melon seeds (ratio 50 : 40 : 10). Values presented are averages of two replicates.

Fermentation time (h)	Moisture content (%)	pH	Water-soluble solids	Total crude protein	Water-soluble protein	Non-protein nitrogen
			(% DWB)			
0	72.69±1.18[a]	6.64±0.04[a]	4.25±0.58[a]	17.65±0.17[a]	ND	ND
12	70.73±0.58[b]	4.80±0.05[b]	2.69±0.15[b]	15.82±0.14[a]	ND	ND
24	72.83±1.54[a]	4.48±0.06[c]	2.73±0.37[b]	18.39±0.21[a]	ND	ND
36	71.86±1.03[a]	4.45±0.05[c]	2.21±0.22[b]	17.63±0.14[a]	ND	ND
48	69.84±0.78[c]	4.42±0.03[c]	1.86±0.14[b]	16.54±0.35[a]	ND	ND

DWB = Dry weight basis.
ND = Not detected.
Means in a column followed by different letters are significantly different (P<0.05).

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 g TIA kg^{-1} DM after 24 and 48 hr incubation, respectively. In fungal fermentation, the significant reduction observed thereafter suggested the production of an L-Dopa-degrading enzyme. However, further work is needed to assess this assumption. In *Mucuna* condiment fermentation, the rapid change in pH towards the alkaline environment (Table 2) might explain the decrease in L-Dopa and the deep darker colour of the 48 hr-fermented condiment. Siddhuraju and Becker (2001) 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.

Table 5. Effect of pre-treatments on L-Dopa levels of velvet bean (% w/w). Values are averages of two replicates, with percentage reduction from raw bean presented in parenthesis

Pre-treatments	% L- dopa (w/w)
Raw bean	6.360 ± 0.010
Boiled, 45min & dehulled	4.713 ± 0.010 (-25. 89%)
Boiled, 45min; dehulled & soaked, 12 h	2.297 ± 0.065 (-63.88%)
Boiled, 45min; dehulled; soaked, 12 h & resoaked, 12 h	1.359 ± 0.071 (78.63%)
Boiled, 45min; dehulled; soaked, 12 h; resoaked, 12 h & reboiled, 45 min	0.639 ± 0.015 (89.95%)

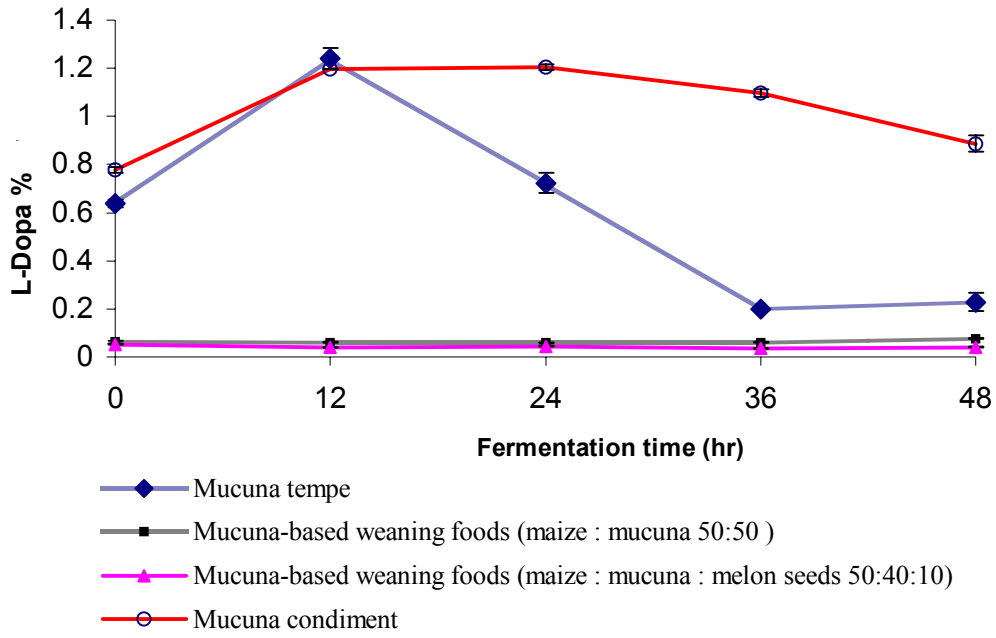

Figure 2. L-Dopa levels (% w/w) in fermented *Mucuna* - based foods.

CONCLUSION

Pre-treatments of the bean before fermentation were the most effective methods for the reduction of L-Dopa in velvet bean. The combination of cooking, dehulling, soaking for 24 hr with removal of soaking water after 12 hr and cooking again reduced approximately 90% of L-Dopa. Further reduction was obtained during fungal fermentation suggesting the production of an L-Dopa-degrading enzyme. All the fermented products contained low levels of L-Dopa (often <0.1%).

Apart from showing that prepared *Mucuna* grains are a good substrate for fungal or natural basic fermentation, these two fermentation techniques improved the bio-availability of nutrients (amino acids) of velvet bean**.** Although this was not as clearly expressed in the natural lactic acid fermentation, the production of *Mucuna*-fortified weaning foods is recommended at household level as a way to alleviate protein-energy malnutrition. Similarly, production of *Mucuna* tempe is recommended. However, large-scale productions are limited by the dehulling process.

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