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Effect of Sonication and Two Solvent Extraction Methods on the L-dopa Concentration and Nutritional Value of *Mucuna pruriens*

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ABSTRACT.

Mucuna pruriens beans are high in crude protein (CP; 25-30%) and starch (39-41%), but also contain toxic L-Dopa. Methods to reduce L-Dopa to a safe threshold (< 0.4%) are often costly and little is known about their impact on the nutritional value of the bean. The objective of this study was to examine effects of three extraction methods on L-Dopa concentration and nutritional composition of finely (1 mm) or coarsely (6 mm) ground beans. Methods evaluated included extraction in solutions of acetic acid (ACD, pH 3) or sodium hydroxide (ALK, pH 11) for 8 hours or sonication (SON) for 5 minutes. All extraction methods decreased the L-Dopa concentration of fine Mucuna particles from 2.8 to < 0.2%, decreased their CP and water-soluble carbohydrate (WSC) concentrations by 24-31% and 78-81%, respectively and increased their NDF and starch concentrations by at least 62 and 14%, respectively. Sonication reduced the ether extract (EE) concentration of fine particles from 5.5% to 4.2% but ACD and ALK did not. Sonication and ACD did not reduce the L-Dopa concentration of coarsely ground beans but ALK reduced it from 2.8 to 2%. Sonication also reduced CP, WSC, and ether EE concentrations of coarse particles by 6, 17, and 27%, respectively and ALK increased their starch concentration by 17%. Therefore, the extraction methods reduced L-Dopa of fine Mucuna particles to safe levels but increased their NDF and starch concentrations at the expense of WSC and CP concentrations. Extraction methods were less effective at reducing the L-Dopa in coarse particles and had fewer, less consistent effects on their nutritional composition.

KEYWORDS: *Mucuna pruriens*, solvent-extraction, sonication

INTRODUCTION

Malnutrition in developing countries is due in part to insufficient access to affordable protein sources. Diets of many children in such countries lack protein and instead consist mainly of cereal-based porridge that is bulky, low in energy and nutrients, and high in antinutrient concentration (Adebowale et al., 2005a). *Mucuna pruriens*, a legume indigenous to tropical regions, can be used to increase the supply of dietary protein to such children. *Mucuna pruriens* beans are high in nutrients including crude protein (25-38%), starch (39-41%), and fiber (4%; Ezeagu et al., 2003; Adebowale et al., 2005b). Adebowale et al. (2007) reported that the bioavailability and amino acid concentrations of Mucuna protein isolates exceeded levels recommended for humans by Food and Agriculture Organization, World Health Organization, and the United Nations for all amino acids except methionine and cysteine. The lysine concentration of *Mucuna*

is particularly high (Bressani, 2002), therefore *Mucuna* is a valuable supplementary protein source to cereal-based diets, which are known to be lysine deficient. The chemical composition of the beans varies with cultivar, geographical location, maturity at harvest, and bean color (St-Laurent et al., 2002; Ezeagu et al., 2003). However, *Mucuna* contains anti-nutritive factors (ANF) and the most potent and problematic ANF in the *Mucuna* bean is L-Dopa (Ukachukwu et al., 2002), the concentration of which ranges from 3 to 7% on a dry basis (Daxenbichler et al., 1972). Symptoms of *Mucuna* intake in humans and monogastric livestock include reduced feed intake, weight loss, diarrhea, vomiting, and skin lesions (Del Carmen et al., 2002; Flores et al., 2002; Szabo and Tebbett, 2002).

Some processing techniques can reduce *Mucuna*'s L-Dopa concentration to the safe threshold of < 0.4% (Eilitta et al., 2003). L-Dopa is readily soluble in dilute solutions of hydrochloric acid (Daxenbichler et al., 1972). Acidification of water to pH 3 allows extraction of the L-Dopa in *Mucuna* beans at 1-mm particle size to safe levels in less than 8 hours (Teixeira et al., 2003). However, this treatment could result in protein loss because of the increased protein solubility at pH less than the isoelectric point (pH 4.0-5.0) of *Mucuna* protein (Adebowale et al., 2007). Alkaline conditions may also facilitate inactivation of L-Dopa in *Mucuna* beans. Diallo et al. (2002) reported that a calcium hydroxide solution was more effective than water at removing L-Dopa from Mucuna bean. Soaking the beans in 4% calcium hydroxide solution for 48 hours reduced the L-Dopa concentration to 0.001%. Teixeira et al. (2003) also reported that extraction of *Mucuna* beans (1 mm particle size) in NaOH solution at pH 11 reduced L-Dopa to safe levels (<0.4%) in less than 8 hours. However, Teixeira et al. (2003) and Wanjekeche et al. (2003) reported that melanin is produced when Mucuna L-Dopa is extracted at alkaline pH and this makes the beans black. Melanin has been anecdotally associated with the formation of melanoma in some studies (Dollery, 1999; Letellier et al., 1999; Siple et al., 2000), but no evidence for this association was found in other studies (Weiner et al., 1993; Pfutzner and Przybilla, 1997; Fiala et al., 2002). Nevertheless, the black color of the alkali-extracted bean may reduce its acceptability. According to Wanjekeche, beans cooked in acid solutions are a lighter shade of black than beans cooked in alkaline solution, therefore they may be viewed as more acceptable. Effective L-Dopa detoxification methods that do not adversely affect the color or nutritional value of the bean are needed. Sonication is a method used in more recent laboratory L-Dopa extraction procedures and it is not associated with discoloration of the beans. St-Laurent et al. (2002) reported 5 minutes to be the most effective duration for sonication. However, effects of sonication on the nutritive value of Mucuna are unknown. Successful removal of L-Dopa from Mucuna beans with solvents depends on the particle size; smaller particles generally increase the surface area and the solid-liquid interaction, promoting the rate of L-Dopa transfer (Teixeira et al., 2003). In contrast, larger particles require less preparation and less expensive equipment such as grinders.

The objective of this study was to examine the effects of the method of extraction on the L-Dopa concentration and nutritional composition of finely (1 mm) or coarsely (6 mm) ground *Mucuna* beans. Methods examined included extraction in either acetic acid (pH 3) or sodium hydroxide (pH 11) for 8 hours or extraction by sonication (SON) in water (pH 7) for 5 minutes.

MATERIALS AND METHODS

Extraction methods

Mucuna pruriens cv. Georgia bush beans were crushed (Roller Mill model 10004, Peerless International, Missouri, USA) and either sieved to pass through a 6-mm screen (USA Standard Testing Sieve, Fisher Scientific, Pittsburgh, PA, USA) or ground in a Wiley mill to pass through a 1-mm screen (Arthur H. Thomas Company, Philadelphia, PA, USA). Twenty-four representative 50-g samples (8 per treatment) of fine (1 mm) or coarse (6 mm) particles were subjected to sonication (SON) in water (neutral pH) or soaked in acidic (ACD) or alkaline (ALK) solutions. The ACD solution was brought to pH 3 by diluting 0.8 ml of a 25% (v/v) acetic acid solution in 2 L of distilled water. The alkaline solution was brought to pH 11 by dissolving 0.1 g of sodium hydroxide in 2 L of distilled water. Each solution was shaken (Eberbach shaker, Michigan, USA) at room temperature for 8 hours (Figure 2), then sieved through four layers of cheesecloth and a Whatman #1 filter paper (1001-240, Fisher Scientific, Pittsburgh, PA, USA). The residue was subsequently rinsed with 1 liter of distilled-deionized water. Samples were also submerged in 2 L of water (pH 7.3) within a sonication bath (Branson Ultrasonics, Connecticut) and sonicated for 5 minutes at room temperature. For each treatment, pairs of replicate residues were composited to provide sufficient sample for chemical analysis (n=4).

Chemical analysis

Sonicated and solvent-extracted residues were dried at 55 oC to 97% DM and ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Dry matter concentration was determined after drying at 60oC for 72 hours and ash was measured by combustion in a muffle furnace at 550oC overnight. Gross energy levels were determined by an adiabatic bomb calorimeter (1261 isoperibol bomb calorimeter, Parr Instrument Company, Moline, Illinois, USA), using benzoic acid as a standard. The following analyses were also conducted: NDF (Van Soest et al., 1991), EE (AOAC, 1984) , WSC (MAFF, 1986), L-Dopa (Siddhuraju and Becker, 2001), CP (Noel and Hambleton, 1976), and starch (Hall, 2001).

Statistical analysis

The experiment had a completely randomized design involving 7 treatments: untreated control, and acid, alkali, or sonication treatments of 1- and 6-mm particle size beans. Each treatment had 4 replicates (n=4) and all values reported are least squares means. Data were analyzed with the MIXED procedure (SAS 9.1, SAS Inst. Inc., Cary, NC, USA). Significance was declared at $P < 0.05$ and means were separated with a PDIFF statement.

RESULTS

All processing methods reduced L-Dopa concentrations of fine Mucuna particles from 2.8% to less than 0.2% (Figure 1). Acid and alkali treatments made the solvents and extracted bean residues darker (Figure 2). All methods also reduced CP and WSC of fine particles by 24-31% and 78-81%, respectively (Table 1) and increased their NDF and starch concentrations by at least 62 and 14%, respectively. Fat concentration of fine particles was reduced from 5.5% to 4.2% by SON, whereas ACD and ALK reduced their

GE values by approximately 10%. The ash concentration of fine particles was increased by 88% and 35% by ALK and SON, respectively.

Sonication and ACD did not reduce L-Dopa concentration of coarsely ground beans but ALK reduced it from 2.8% to 2%. Sonication reduced CP, WSC, and fat concentration of coarse particles by 6, 17, and 27%, respectively. The ALK treatment increased their starch concentration by 17% but decreased their WSC concentration by 78%. The ACD treatment increased the NDF concentration of coarse particles by 35% but decreased their WSC and fat concentrations by 51% and 31%. Ash concentration and GE of coarse particles were not affected by any of the treatments.

Table 1. Effect of processing method on the chemical composition of fine (1 mm) and coarse (6 mm) *Mucuna* beans

Item	Unground ControlA	ACDB 1 mm	ALKC 1 mm	SOND 1 mm	ACDB 6 mm	ALKC 6 mm	SOND 6 mm	SEM
Dry matter, %	95.4	94.9	95.3	95.6	95.1	95.5	95.1	0.2
Crude protein, % DM	25.4a	19.3c	17.9cd	17.4d	25.3ab	24.6ab	23.9b	0.5
Ash, % DM	6.0c	7.9bc	11.3a	8.1b	6.6bc	6.9bc	7.3bc	0.7
Gross energy, Kcal/g	4.1a	3.6b	3.6b	3.8ab	4.0ab	3.9ab	3.9ab	0.13
Starch, % DM	38.2b	45.9a	46.2a	43.7a	36.8b	44.8a	34.6b	1.5
WSC, % DM	18.1 a	3.8 d	3.9 d	3.5 d	8.8 c	13.3b	15.0b	0.8
Fat, % DM	5.5a	5.9a	5.6a	4.2b	3.8c	3.6c	4.0b	0.4
NDF, % DM	17.3 e	32.0 b	38.0a	28.1bc	23.4c	21.0de	20.3de	1.9

Within a row, means without a common superscript letter differ ($P < 0.05$); A untreated beans; B acid-treated beans; C alkali-treated beans; D sonicated beans; WSC = water-soluble carbohydrate; NDF = neutral detergent fiber.

Figure 1. Effect of acid extraction (ACD), alkali extraction (ALK), or sonication (SON on the L-Dopa concentration of fine (1 mm) or coarse (6 mm) Mucuna particles. Means without common letters differ ($P < 0.05$); error bars denote standard error.

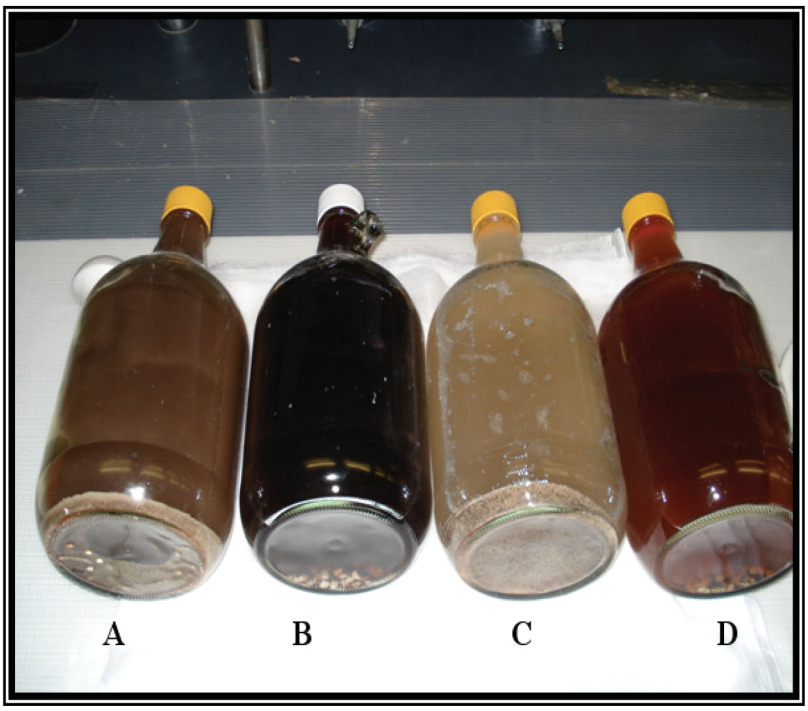
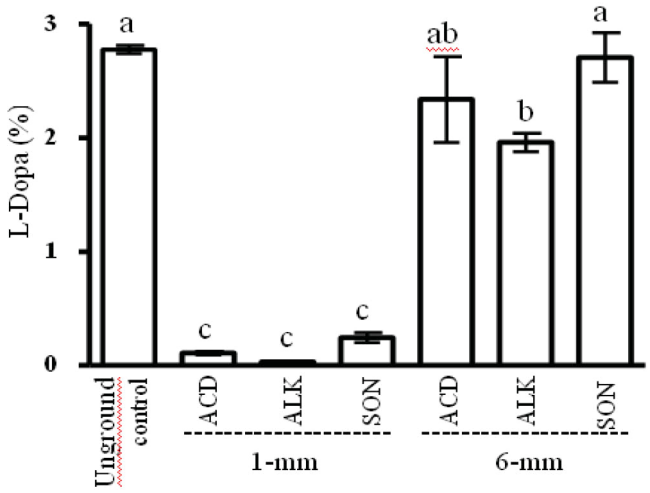


Figure 2. Color changes after detoxification of *Mucuna* bean through A) Alkaline extraction at 1-mm particle size, B) Alkaline extraction at 6-mm particle size, C) Acid extraction at 1-mm particle size, D) Acid extraction at 6-mm particle size.



DISCUSSION

Safe L-Dopa levels in Mucuna beans destined for monogastric livestock consumption are considered to be 0.4% or less (Eilitta et al., 2003). All extraction methods were equally effective in reducing the L-Dopa concentration of fine Mucuna particles to < 0.2% and thus making them safe for consumption by monogastrics. However, the L-Dopa concentration of coarse Mucuna particles was not decreased by ACD and SON and the 29% reduction by ALK treatment was inadequate to make the bean safe for consumption by monogastrics. These particle-size dependent responses are in agreement with Teixeira et al. (2003), who showed that L-Dopa removal at pH 3 or 11

depended on particle size; it was more effective in beans ground to a 1-mm particle size versus that ground to 2-, 4-, and 8-mm sizes. The efficacy of the acid and alkali-treatment of fine particles also agrees with the observations of Teixeira et al. (2003).

The CP concentration of fine particles was reduced by 24-31% in all treatments but that of coarse particles was only reduced by SON. The latter was likely because of the cell rupturing effect of sonication, which would have exposed more of the protein to the solvent and thus increased solvent penetration. Most (67.5%) of the protein in Mucuna is water-soluble (Adebowale et al., 2007). Greater CP and WSC losses in finer particles were because of the greater surface area exposure. This is in agreement with Myhrman (2002) and Teixeira et al. (2003) who reported CP losses of 11% and up to 50%, respectively due to leaching after soaking of finely ground bean samples in acid or alkaline solutions. Losses of CP from ACD and ALK-treated fine particles were also facilitated by the solubility of Mucuna protein. For unknown reasons, the alkaline extraction of Mucuna did not lead to greater CP losses than the acidic extraction. More work should be done on effects of these extraction methods on the true protein concentration of Mucuna.

The increased starch concentration of fine particles due to ACD, BAS or SON treatments of fine particles agrees with responses to acid or alkali extraction of Mucuna reported by Siddhuraju and Becker (2005). The increased starch concentration was due to partial loss of soluble components including WSC, protein, and L-Dopa, all of which decreased with solvent extraction relative to CON. The reduction in concentration of these components and the energy value of the bean, and the concomitant increases in starch and fiber concentration imply that solvent extraction and sonication modified the nutritive value of the bean and resulted in losses of key components. Further research should determine effects of the processing methods employed in this experiment on concentrations of amino acids in Mucuna.

The pH of the solvent is an important factor that can affect the success of L-Dopa removal from Mucuna and the residual nutritional quality. Several authors mention that due to formation of melanin, Mucuna beans are darker after acid or alkali extraction (Teixeira et al., 2003; Wanjekeche et al., 2003). Melanin is a metabolite of L-Dopa characterized by its dark color and conversion of L-Dopa into melanin is most evident at alkaline pH (Teixeira et al., 2003; Wanjekeche et al., 2003). The darker color of the alkali versus acid extracts in this study (Figure 2) agrees with these observations.

Diallo et al. (2002) successfully reduced the L-Dopa concentration to 0.001% after 48 hours of soaking cracked Mucuna beans in calcium hydroxide solution, but noted the remarkably dark coloration upon treatment. Beans cooked in acid solutions are lighter in color than beans cooked in alkaline solutions (Wanjekeche et al., 2003). Adebowale et al. (2007) noted that darker colors occurred in sodium hydroxide solutions of pH 11 relative to less alkaline solutions. This causes concern because the effects of melanin on health are controversial. Melanin has been anecdotally associated with the formation of melanoma in some studies (Dollery, 1999; Letellier et al., 1999; Siple et al., 2000), but no evidence for this association was found in other studies (Weiner et al., 1993; Pfutzner and Przybilla, 1997; Fiala et al., 2002). Discarding the solvent residue may reduce this concern. Nevertheless, the darker color of the extracted bean indicates the need for further investigation of concentrations of melanin residues in the detoxified bean.

CONCLUSION

Particle size affected the efficacy of L-Dopa removal in the solvent extracts. Both acidic and alkaline solvents performed equally well at detoxifying fine particles of Mucuna bean to safe levels (<0.4% L-Dopa) but also reduced their WSC and CP concentrations and increased their starch and NDF concentrations. However, these methods were not effective at detoxifying coarse Mucuna particles and they had less consistent effects on their nutritive value. Acidic and alkaline solvent extraction darkened the bean, suggesting that they increased the formation of melanin, a metabolite of L-Dopa characterized by its dark color. Future research should determine melanin concentrations in acid- or alkali-extracted beans as well as their concentrations of true protein and amino acids.

Sonication did not cause discoloration of Mucuna , yet it was also an effective method of detoxifying fine but not coarse particles of Mucuna to safe levels (<0.4% L-Dopa). Sonication generally resulted in similar modifications to the nutritive value of the bean as acid or alkali solvent extraction but caused greater losses of fat from fine particles.

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