THE EFFECT OF *Mucuna pruriens* DETOXIFICATION, THROUGH SONICATION AND ALKALI OR ACID EXTRACTION, ON L-DOPA CONCENTRATION AND NUTRITIONAL VALUE[†]

[EL EFECTO DE LA DESTOXIFICACIÓN DE *Mucuna pruriens*, A TRAVÉS DE LA SONICACIÓN Y LA EXTRACCIÓN ALCALINA O ÁCIDA, SOBRE LA CONCENTRACIÓN L-DOPA Y EL VALOR NUTRICIONAL]

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SUMMARY

Mucuna pruriens is a high-protein food and feed and a nutraceutical; however, its L-Dopa content limits its use for these purposes. Effects of 3 extraction methods on L-Dopa concentration and nutritional composition were determined. Beans were ground to 1 mm (Fine) or 6 mm (Coarse) and divided into 3 treatments (n=8 samples/treatment): sonication (SON) for 5 minutes in water or extraction in solutions of 25 g/100g acetic acid (ACD, pH 3) or sodium hydroxide (ALK, pH 11) for 8 hours. Fine particle treatments decreased (P<0.05) concentrations of L-Dopa (from 2.8 to <0.2 g/100g), crude protein (CP) and water-soluble carbohydrates (WSC) and increased (P<0.05) their aNDF and starch concentrations; sonication reduced (P<0.05) the ether extract (EE). Among coarse particles, only ALK reduced (P<0.05) the L-Dopa concentration (from 2.8 to 2 g/100g). Sonication reduced (P<0.05) the CP, WSC, and EE concentrations and ALK increased (P<0.05) their starch concentration. Acid extraction consistently increased (P<0.05) all amino acid concentrations. Alkali extraction also increased (P<0.05) concentrations of amino acids except lysine. However, sonication decreased (P<0.05) concentrations of amino acids except cysteine and proline. In conclusion, the most effective extraction methods reduced L-Dopa in fine *Mucuna* particles to safe levels, but increased aNDF and starch concentrations while reducing WSC and CP.

Keywords: *Mucuna pruriens*; Velvet beans; Solvent-extraction; Sonication; Acid; Alkali

RESUMEN

Mucuna pruriens es una fuente de alimento y nutracéutico; sin embargo, su contenido de L-Dopa disminuye su potencial total. Se determinaron los efectos de 3 métodos de extracción sobre la concentración de L-Dopa y la composición nutricional. Los granos se molieron a 1 mm (Fino) o 6 mm (Grueso) y se dividieron en 3 tratamientos (n = 8): sonicación (SON) durante 5 minutos en agua o extracción en soluciones de 25 g/100 g de ácido acético (DCA, pH 3) o hidróxido de sodio (ALK, pH 11) durante 8 horas. Los tratamientos con partículas finas disminuyeron las concentraciones de L-Dopa (de 2.8 a < 0.2 g/100g), proteína cruda (PC) y carbohidratos solubles en agua (CSA) y aumentaron sus concentraciones de aFDN y almidón; la sonicación redujo el extracto etéreo (EE). Entre las partículas gruesas, solo ALK redujo la concentración de L-Dopa (de 2.8 a 2 g/100g). La extracción de ácido aumentó constantemente todas las concentraciones de aminoácidos. La extracción de álcali aumentó las concentraciones de aminoácidos excepto lisina. En conclusión, los métodos de extracción más efectivos redujeron la concentración de L-Dopa de todas las partículas finas de mucuna a niveles seguros, pero aumentaron las concentraciones de aFDN y almidón.

Palabras clave: *Mucuna pruriens*; Frijoles Velvet; Extracción-solvente; Sonicación; Ácido; Álcali.

INTRODUCTION

Mucuna pruriens, a legume indigenous to tropical regions, can be used as a supplemental protein source in the diets of animals and people; it also has nutraceutical and anti-nutrient properties (Rai *et al*., 2017; Mohan *et al*., 2016). *Mucuna pruriens* beans have high concentrations of crude protein (CP, 25-38 g/100g) and starch (39-41 g/100g) (Adebowale *et al*., 2005; Ezeagu *et al*., 2003) and the bioavailability and amino acid concentrations of *Mucuna* protein isolates exceed levels recommended for humans by the Food and Agriculture Organization, the World Health Organization, and the United Nations for all amino acids except methionine and cysteine (Adebowale *et al*., 2007). The lysine concentration of *Mucuna* is particularly high (Bressani, 2002), therefore *Mucuna* is potentially a valuable supplementary protein source to cereal-based diets, which are known to be lysine deficient. However, *Mucuna* contains anti-nutritive factors (Mohan *et al*., 2016) the most potent of which is L-Dopa (Ukuchkwu *et al*., 2002). The concentration of *Mucuna* L-Dopa ranges from 3 to 7 g/100g on a dry basis (Szabo and Tebbett, 2002). 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; Szabo and Tebbett, 2002).

Some processing techniques can reduce *Mucuna* L-Dopa concentration to the safe threshold of <0.4 g/100g (Eilitta *et al*., 2003). *Mucuna* 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). Ensiling *Mucuna* beans for 28 days reduced the L-Dopa concentration by 43 to 61%, but the concentration of residual L-Dopa in the bean exceeded safe levels (Huisden *et al*., 2014). 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 g/100g calcium hydroxide solution for 48 hours reduced the L-Dopa concentration to 0.001 g/100g. 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 g/100g) 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 (St-Laurent *et al*., 2002; Spence, 2018). Sonication is a method used in more recent laboratory L-Dopa extraction procedures and it does not discolor 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 with smaller particles generally promoting the rate of L-Dopa removal to a greater extent (Teixeira *et al*., 2003).

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. This study is the second in a series of studies (Huisden *et al*., 2010, 2014 and 2018) aimed at evaluating the efficacy of *Mucuna* detoxification methods and their effects on the nutritive value of the detoxified bean.

MATERIALS AND METHODS

About 370 kg of *Mucuna pruriens*, cv. preto beans were harvested from a 10 ha field in Sao Paolo, Brazil and used for this study and the other studies in this series (Huisden *et al*., 2014, 2018) and the sheep deworming study of Huisden *et al*. (2010). The beans contained 25 g/100g CP, 4.6 g/100g EE, 17.3 g/100g aNDF, 18.1 g/100g WSC, 38.2 g/100g starch, and 2.8 g/100g L-Dopa.

Extraction methods. The 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). Of 24 representative 50-g samples of fine (1 mm) and coarse (6 mm) particles, each of 8 replicate samples was individually 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 adding 25 ml/100ml (v/v) acetic acid solution to distilled water, up to a final volume of 2 L. The alkaline solution of pH 11 was produced by adding sodium hydroxide to distilled water, up to a final volume of 2L. Each replicate suspension was shaken (Eberbach shaker, Michigan, USA) at room temperature for 8 hours, then sieved

through four layers of cheesecloth and a Whatman #1 filter paper (1001-240, Fisher Scientific, Pittsburgh, PA, USA). The residues were subsequently rinsed with 1 L of distilled-deionized water. Eight replicate samples were also submerged in 2 L of water (pH 7) within a sonication bath (Branson Ultrasonics, Connecticut) and sonicated for 5 minutes at room temperature. For each treatment, pairs of replicate residues were composited for chemical analysis (n=4).

Chemical analysis. Sonicated and solvent-extracted residues were dried at 55 ºC to constant weight 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 60 ºC for 72 hours and ash was measured by combustion in a muffle furnace at 550 ºC overnight. Gross energy was determined with 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: aNDF (Van Soest *et al.*, 1991), EE (AOAC, 1984; Method 24.005), WSC (MAFF, 1986), L-Dopa (Siddhuraju and Becker, 2001), CP (Noel and Hambleton, 1976), amino acids (AOAC, 1994; Method 994.12) and starch (Hall, 2001). Tannin concentration quantification was based on the Butanol-HCl procedure of Terrill *et al.* (1992) and the Radial Diffusion Assay for Tannins (Hagerman, 1987). Purified quebracho tannin extract was used as the standard and results were expressed as quebracho tannin equivalents.

Statistical analysis. The experiment had a 2 (particle sizes) × 3 (extraction methods) factorial treatment structure with a control and it was analyzed as a completely randomized design with 4 replicates per treatment. Data were analyzed with the MIXED procedure (SAS, 2005) and a model containing treatment effects. Significance was declared at P<0.05 and means were separated with Fisher's F-protected least significant different test. This approach was used instead of contrasts to detect differences between all particle sizes by extraction method combinations. Means reported are least square means.

RESULTS

All processing methods reduced (P<0.05) L-Dopa concentrations of fine *Mucuna* particles from 2.8 g/100g to less than 0.2 g/100g (Figure 1).

Acid and alkali treatments made the solvents and extracted bean residues darker (Figure 2). All methods also reduced (P<0.05) CP and WSC concentrations of fine particles by 24-31% and 78-81%, respectively

(Table 1) and increased (P<0.05) their aNDF and starch concentrations by at least 62 and 14%, respectively. Ether extract concentration of fine particles was reduced (P<0.05) from 5.5 g/100g to 4.2 g/100g by SON, whereas ACD and ALK reduced (P<0.05) GE values of fine particles by approximately 10%. The ash concentration of fine particles was increased (P<0.05) by 88% and 35% by ALK and SON, respectively. No tannins were detected in the *Mucuna* bean samples with either of the analytical methods that were used.
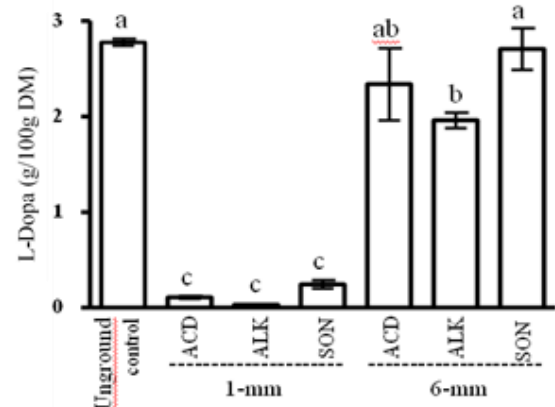

Figure 1. Concentration of L-Dopa after the various treatments; ($P < 0.05$) and error bars denote standard error.
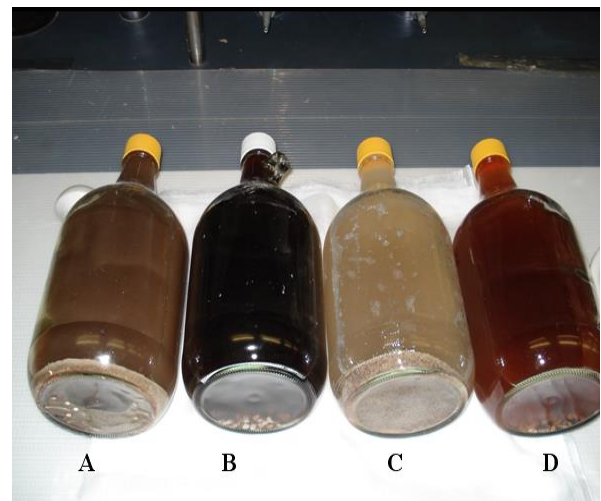

Figure 2. Color changes after detoxification of *Mucuna* bean through A) Alkaline extraction at 1-mm particle size, B) Alkaline extraction at 6-mm particle size, C) Acid extraction at 1-mm particle size, D) Acid extraction at 6-mm particle size.

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

Item	Control[A]	Fine			Coarse			
		ACD[B]	ALK[C]	SON[D]	ACD[B]	ALK[C]	SON[D]	SEM
Dry matter, g/100g	95.4	94.9	95.3	95.6	95.1	95.5	95.1	0.2
Crude protein, g/100g DM	25.4[a]	19.3[c]	17.9[cd]	17.4[d]	25.3[ab]	24.6[ab]	23.9[b]	0.5
Ash, g/100g DM	6.0[c]	7.9[bc]	11.3[a]	8.1[b]	6.6[bc]	6.9[bc]	7.3[bc]	0.7
Gross energy, MJ/kg DM	17.1[a]	15.1[b]	15.1[b]	15.9[ab]	16.7[ab]	16.3[ab]	16.3[ab]	0.54
Starch, g/100g DM	38.2[b]	45.9[a]	46.2[a]	43.7[a]	36.8[b]	44.8[a]	34.6[b]	1.5
WSC, g/100g DM	18.1[a]	3.8[d]	3.9[d]	3.5[d]	8.8[c]	13.3[b]	15.0[b]	0.8
EE, g/100g DM	5.5[a]	5.9[a]	5.6[a]	4.2[b]	3.8[c]	3.6[c]	4.0[b]	0.4
aNDF, g/100g DM	17.3[e]	32.0[b]	38.0[a]	28.1[bc]	23.4[c]	21.0[de]	20.3[de]	1.9

[a-e] 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; aNDF = neutral detergent fiber.

Acid extraction consistently increased (P<0.05) the amino acid concentrations of the finely ground beans and resulted in the greatest (P<0.05) values. Compared to the control, alkali extraction also increased (P<0.05) concentrations of all amino acids except lysine. However, sonication decreased (P<0.05) concentrations of all amino acids except cysteine and proline (Table 2).

Sonication and ACD did not reduce (P>0.05) L-Dopa concentration of coarsely ground beans but ALK reduced (P<0.05) the values from 2.8 to 2 g/100g. Sonication reduced (P<0.05) CP, WSC, and EE concentration of coarse particles by 6, 17, and 27%, respectively. The ALK treatment increased (P<0.05) starch concentration of Coarse particles by 17% but decreased (P<0.05) their WSC concentration by 78%. The ACD treatment increased (P<0.05) the aNDF concentration of coarse particles by 35% but decreased (P<0.05) their WSC and EE concentrations by 51% and 31%. Ash concentration and GE of coarse particles were not affected (P>0.05) by any of the treatments. The amino acid profile of coarsely ground *Mucuna* was not measured because the detoxification methods had little effect on the L-Dopa concentration of the coarsely ground beans.

DISCUSSION

Removal of anti-nutrient properties, such as L-Dopa, may be crucial to the safe use of legumes as a food source (Mohan *et al.*, 2016). Safe L-Dopa levels in *Mucuna* beans destined for monogastric livestock consumption are considered to be 0.4 g/100g 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 g/100g 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 those ground to 2-, 4-, and 8-mm sizes. The efficacy of the ACD and ALK-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% by all detoxification methods 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 solubilization.

Most (67.5 g/100g) 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 Crude protein losses due to increased solubility at low and high pH (Adebowale *et al.*, 2007) are consistent with our findings of CP loss with the ACD and ALK treatments. Other than discoloration, no precipitation, haze or clogging of filter pores was evident observed in the beans.

Sonication may have decreased the amino acid concentration of the beans because its' cell rupturing effect may have increased their solubility. The contrasting increases in concentrations of amino acids in ACD- and ALK-treated beans are probably evidence of increased proteolysis and peptidolysis by these treatments.

The increased starch concentration of fine particles due to ACD, ALK or SON treatments 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 composition of the bean and resulted in losses of key components. Nevertheless, except for SON, the extraction methods did not reduce the concentration of amino acids in the beans.

Table 2. Effect of processing method on the amino acid composition of finely ground (1-mm) *Mucuna* beans, DM basis.

Item	Control[A]	ACD[B]	ALK[C]	SON[D]	SEM
Methionine	0.22[c]	0.25[a]	0.24[b]	0.20[d]	0.002
Cysteine	0.25[c]	0.30[a]	0.27[b]	0.25[c]	0.003
Lysine	1.12[b]	1.20[a]	1.15[b]	1.05[c]	0.012
Threonine	0.75[c]	0.87[a]	0.80[b]	0.70[d]	0.007
Arginine	1.06[c]	1.23[a]	1.12[b]	0.99[d]	0.008
Isoleucine	0.79[c]	0.96[a]	0.89[b]	0.75[d]	0.005
Leucine	1.24[c]	1.5[a]	1.39[b]	1.16[d]	0.009
Valine	0.86[c]	1.05[a]	0.98[b]	0.83[d]	0.007
Histidine	0.43[c]	0.51[a]	0.46[b]	0.41[d]	0.003
Phenylalanine	0.83[c]	0.99[a]	0.92[b]	0.78[d]	0.007
Glycine	0.79[c]	0.94[a]	0.86[b]	0.74[d]	0.005
Serine	0.80[c]	0.94[a]	0.87[b]	0.74[d]	0.007
Proline	0.86[c]	1.03[a]	0.94[b]	0.84[c]	0.008
Alanine	0.66[c]	0.79[a]	0.73[b]	0.62[d]	0.005
Asparagine	1.99[c]	2.32[a]	2.12[b]	1.88[d]	0.012
Glutamine	2.05[c]	2.34[a]	2.18[b]	1.94[d]	0.015

[a-d] 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.

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 mentioned 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 g/100g after 48 hours of soaking cracked *Mucuna* beans in calcium hydroxide solution but noted the remarkably dark coloration of the treated beans. 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. The association of such dark colorations with melanin is should be investigated because the effects of melanin on health are controversial (Ziyang *et al.*, 2018). Melanin has been anecdotally associated with the formation of melanoma in some studies (Dollery, 1999; Letellier *et al.*, 1999; Siple *et al.*, 2000) but not others (Weiner *et al.*, 1993; Pfutzner and Przybilla, 1997; Fiala *et al.*, 2002). The darker color of the solvent-extracted bean in this study highlights the need for further investigation of concentrations of melanin residues in the acid- or alkali-treated bean. The effect of these treatments on *Mucuna's* food or feed safety (Huisden *et al.*, 2018) and nutraceutical properties (Rai *et al.*, 2017; Tavares *et al.*, 2017; Mohamed *et al.*, 2018;), also require further investigation; especially since it has been suggested that L-Dopa plays a role in its ethnopharmacological effects (Huisden *et al.*, 2010).

CONCLUSIONS

Particle size affected the efficacy of L-Dopa removal in the solvent extracts. Both acidic and alkaline solvents performed equally well at detoxifying fine (1 mm) particles of *Mucuna* bean to safe levels (<0.4 g/100g L-Dopa) but also reduced their WSC and CP concentrations and increased their starch and aNDF concentrations. However, these methods were not effective at detoxifying coarse (6 mm) *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.

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 g/100g 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 EE from fine particles and unlike the other treatments, it decreased the concentrations of the amino acids.

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