Antinutrients in the cassava (*Manihot esculenta* Crantz) leaf powder at three ages of the plant

Antinutrientes na farinha de folhas de mandioca (Manihot esculenta Crantz) em três idades da planta

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Abstract

In Brazil, the cassava leaf meal (CLM) has been used to strive against undernourishment because it is a high source of vitamins and minerals. However, the wide variation in the chemical composition of the different cultivars, as well as their antinutritional substances may be a restriction to their uses. The levels of some antinutrients in CLM from five cultivars at three ages of the plant (TAP) were investigated, in order to select the cultivars and plant ages that would be more appropriate for human consumption. The lowest contents of antinutrients were observed in the 12-month old plants, except for nitrate and hemagglutinin from which the lowest contents were found for the 17 month old ones. The cultivar IAC 289-70 had the lowest antinutrient levels, except for saponin and oxalate. Thus, the cultivar IAC 289-70 at 12 months is the most appropriate for human consumption.
Keywords: cassava leaves; cyanide; polyphenol; saponin; hemagglutinin; cultivars.

Resumo

No Brasil, a farinha de folhas de mandioca (FFM) vem sendo usada no combate à desnutrição por ser fonte de vitaminas e minerais. Todavia, a grande variação na composição química dos diferentes cultivares e a presença de substâncias antinutricionais tem restringido o seu uso. Investigaram-se os níveis de alguns antinutrientes da FFM de cinco cultivares em três idades da planta (TIP), a fim de selecionar cultivares e idades mais adequadas para o consumo humano. Aos 12 meses foram observados os menores teores de antinutrientes, exceto para nitrato e hemaglutinina, os quais foram inferiores aos 17 meses. O cultivar IAC 289-70 destacou-se com os menores níveis dos antinutrientes, exceto para a saponina e oxalato. Portanto, o cultivar IAC 289-70, aos 12 meses, é o mais adequado para o consumo humano.
Palavras-chave: folha de mandioca; cianeto; polifenóis; saponina; hemaglutinina; cultivares.

1 Introduction

Undernourishment gives rise to different sequelae in the world population, as it is the main cause of the high rates in infant mortality throughout the developing countries[28]. To strive against the problem or to weaken it, some Brazilian non-government organizations have used the cassava leaf meal (CLM) either as one of the "multimistura" components[4,27,41] or by adding it in small portions into school snacks[31]. These and other initiatives have led to significant decreases in the undernourished rates in the country over the last two decades[45].

Cassava roots are widely used and represent the basic diet of about 500 million people in the world[8]. The use of the CLM might supply a positive balance for the nutritional quality because they present higher contents of proteins, vitamins, minerals and fiber[14,34]. In addition, their low production cost is emphasized, since the leaves are rather considered as residues and they do not compete with the roots, which are the main commercial product from cassava[40].

Although CLM is a source of fibers, vitamins and mineral, it also has some antinutritional and toxic substances. These substances interfere with digestibility and uptake of the nutrients, and they might present toxic effects depending on the amount in which they are consumed. However, their consumption at low amounts may even bring about some benefits to the human organism. An example is found in the antioxidant action and anticarcinogenic properties of the polyphenols[1,9,10,30,33].

The wide variation in the chemical composition of the leaves in different cassava cultivars is a restrictive factor to their use as an alternative food source to the population. The cyanide is the main toxic factor restricting the use of CLM, which presents a wide variation range from 5.3 to 80 mg.100 g^{-1} dry matter (DM)[3,6,21,37,39]. Few data related to the influence of the plant age on the contents of the CLM antinutrients are registered in the available literature. The polyphenols and cyanide have been quite well investigated, but only few reports on the other antinutritional factors are available, and no information about hemagglutinin has been found. Therefore, in the present study the contents of some CLM antinutrients – oxalate, nitrate, cyanide, polyphenol, hemagglutinin, trypsin inhibitor, saponin – in five cultivars at three ages of the plant (TAP) were determined in order to select the cultivar and their more appropriate ages of harvesting for human consumption.

2 Materials and methods

2.1 Collecting the leaves and preparing the samples

The ripe cassava leaves of five cultivars Ouro do Vale, Maracanã, Mantiqueira IAC 24-2 (MANT.IAC24-2), IAC 289-70 and Mocotó from the experimental area pertaining to the UFLA

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Agricultural Department were collected, at TAP, in the morning in October/2001 (12 months), January/2002 (15 months) and March/2002 (17 months).

The collected leaves were transported in plastic bags to the drying place and were dried in the shade in a closed and airy room at environmental temperature. On the 10th day of drying, as the leaves of the first collection were still too moist for milling, they were transferred to a ventilated oven at 30 °C for 90 minutes. This procedure was also used for the other collections. Then, the leaves were triturated (without petiole) in a mill provided with a 40-mesh sieve. The meals were stored in glass recipients and protected from light until the analyses were performed at the Biochemistry Laboratory belonging to the Universidade Federal de Lavras (UFLA) Chemical Department.

2.2 Registration of the pluviometric index

The pluviometer index from the planting time (October/2000) until the last collection of the leaves (March/2002) was obtained at Coronel Roberto Venerando Pereira Climatological Station, belonging to the 5th INMET Meteorology District, located at the UFLA campus.

2.3 Physical, chemical and statistical analyses

Moisture was determined in both the fresh leaves and CLM[2], but calcium contents were determined only in the CLM[29]. The oxalic acid was determined by a titration method[26]. Colorimetric methods were used for Nitrate[7] and polyphenol[20]. The preparation of the linamarase enzymatic extract and the quantification of cyanide in the samples were done[13].

The estimate of the agglutinative activity of the CLM extracts were carried out[18] with some modifications. A suspension of 2% erythrocytes was used (human blood A, Rh^+) and added to the extract of the sample, carrying out a series of dilutions on the base 2 (2^0, 2^1, 2^2, 2^3, etc.). The agglutinative titer was visually determined and the results were expressed by the basis-2 exponential, corresponding to the last dilution in which the visible erythrocyte agglutinations were observed.

The determination of the trypsin inhibitor in CLM was done by applying the enzymatic/colorimetric method[24,25]. The CLM steroidal saponin were determined by the colorimetric method[4].

The entirely randomized statistical design was used, on a factorial scheme of 5 x 3 x 3, which is, five cultivars, three plant ages, and three replicates. The Tuckey test was used for comparison among the averages at a probability level of 5%[38].

3 Results and discussion

The CLM was prepared by drying the leaves in shade and environmental conditions because this way is the most similar to the one carried out by the population in general. In addition, drying in the shade, in a closed and airy compartment was the condition occasioning the lowest residual levels of cyanide in CLM[13].

The average values for moisture contents in the leaves and CLM ($g.100\ g^{-1}$) were 70.46 ± 1.15 and 9.16 ± 0.81, respectively. In the variance analysis, significant differences were observed at 1 or 5% probability, by the F test, in all other parameters studied.

Table 1 shows the average contents in dry matter (DM) of oxalate, calcium, the ratio between the calcium and oxalate, nitrate and cyanide in CLM of the five cultivars studied at TAP. The lowest oxalate levels were found in the 12-month old cultivars, except for cultivars Ouro do Vale and Maracanã. The Mocotó cv. (12 mo) had the lowest contents, but did not significantly differ from the Maracanã cv.

The variation observed in oxalate contents is within the range reported in the available literature (1.35 to 2.88 $g.100\ g^{-1}$ DM) for CLM from other cultivars[12,19]. A calcium/oxalate ratio below 0.44% in a given food will endanger the uptake of the calcium contained in this food[19]. According to the results, all cultivars at TAP showed a higher calcium/oxalate ratio (0.44 $g.100\ g^{-1}$ DM), therefore the oxalate levels found in CLM do not endanger the uptake of the calcium contained in it. It is worth emphasizing that diets with low calcium and high oxalate concentrations are not recommended. However, the occasional consumption of

Table 1. Average contents of oxalate, calcium, oxalate/calcium ratio, nitrate and cyanide in cassava leaf meal at three ages of the plant.

Cultivars	Oxalate ($g.100\ g^{-1}$ DM)[1]			Calcium ($g.100\ g^{-1}$ DM) + Calcium/oxalate		
	12 mo	15 mo	17 mo	12 mo	15 mo	17 mo
Ouro do Vale	2.48^{bA}	2.22^{cB}	2.86^{aA}	1.95 bA + 0.79	1.89^{bA} + 0.85	2.21 aA + 0.77
Maracanã	1.49^{Bc}	1.61^{aE}	1.37^{cD}	1.20 C + 0.80	1.18^{C} + 0.73	1.15 D + 0.84
MANT.IAC	1.89^{Bb}	2.02^{aC}	1.97^{abC}	1.60 B + 0.85	1.58^{B} + 0.78	1.61 C + 0.82
IAC 289-70	1.94^{bB}	2.47^{aA}	2.53^{aB}	1.56 cB + 0.80	1.67^{bB} + 0.68	1.80 aB + 0.71
Mocotó	1.36^{bC}	1.82^{aD}	1.38^{bD}	0.98 cD + 0.72	1.22^{aC} + 0.67	1.11 bD + 0.80
Cultivars	Nitrate ($mg.100\ g^{-1}$ DM)[2]			Cyanide ($mg.100\ g^{-1}$ DM)		
Ouro do Vale	74.66 ± 0.33	ND[3]	ND	11.29^{cC}	20.93^{bA}	29.22^{aC}
Maracanã	74.30 ± 0.09	ND	ND	19.33^{bA}	18.04^{bB}	35.02^{aA}
MANT.IAC	58.70 ± 0.20	ND	ND	10.80^{cC}	16.03^{bC}	34.51^{aA}
IAC 289-70	43.05 ± 0.24	ND	ND	13.77^{B}	12.46^{D}	12.38^{D}
Mocotó	43.20 ± 0.27	ND	ND	12.53^{cBC}	20.04^{bA}	31.63^{aB}

[1]Averages followed by the same small letters in the lines and capital letter in the columns do not differ from each others (Tukey test $p \leq 0.05$); [2]Data refer to the average ± pattern deviation of three replicates; and [3]ND = non-detected.

foods with high oxalate concentrations, making up a balanced diet, does not seem to present any problems[42].

The nitrate levels decreased with the maturity of the plant, whereas those of cyanide had opposite behavior. The cultivar IAC 289-70 had the lowest contents of nitrate, and at 15- and 17-month old its cyanide levels were the lowest ones. The nitrate contents were inferior to those found by CORRÊA[12] for cassava leaves from the Baiana cv. (160 to 310 mg.100 g^{-1} DM) dried under either the sun or in the shade in an oven at 30 and 40 °C. The differences of levels of this antinutrient may be due to the cultivar itself, the plant age, as well as to soil fertilization, since the application of nitrogen fertilizers and potassium lead to the accumulation of nitrate[46].

The contents of cyanide observed in CLM from all five cultivars at TAP are within the range reported in other work: from 5.3 to 80 mg.100 g^{-1} DM[3,6,21,37,39]. This wide variation is probably due to the genetic differences among the cultivars, the plant ages, the leaf drying temperatures, the leaf maturity, the soil fertility and the pluviometric precipitation index. The influence of the lack of rainfall events on the reduction of cyanide contents is reported[11,21]. For this reason, the data of the pluviometric precipitation were collected from the planting period (October/2000) until the last collection of the leaves (March/2002), and are shown in Figure 1. A heterogeneous distribution of the rainfall was observed, since the drought period preceded the first collection of the leaves, when the plants were 12 months old, and exactly at this plant age the lower contents of cyanide were observed.

The lethal dose (LD) of cyanide oscillates from 0.5 to 3.5 mg.kg^{-1} human body weight[47]. Therefore, for a 70 kg-weighed individual, the maximum reliable consumption is about 110 g CLM from any one of those five cultivars at TAP; and this amount is bulky because the powder presents low density. However, it is worth emphasizing that the chronic toxicity is due to the consumption of lower cyanide doses at longer timer intervals[36].

The CLM from the analyzed cultivars are classified as toxic because generally their cyanide contents are above 10 mg.100 g^{-1} powder[22]. However, considering that the "multimistura" (food supplement) contains 3% CLM[27] and supposing it was prepared by using these cultivars under analysis, the maximum ingested cyanide would be 0.94 mg, which is not a toxic amount. The addition of one teaspoon, approximately 2 g CLM in each meal[31] would also not cause any toxicity problems.

Table 2 shows the polyphenol contents, trypsin inhibitor, saponin and the hemagglutinative activity of CLM from these five cultivars at TAP. In general, it is observed that the polyphenol contents increased with the maturity of the plant, and this tendency was already described[21].

The lowest polyphenol contents were found in Maracanã cv. when the plant was 12 months old, which did not differ significantly from the cultivars Mocotó and IAC 289-70, at the same age. The polyphenol contents found in CLM are according to those reported in the available literature, that is, 2.1 to 120 mg.100 g^{-1} DM[3,6,21,37].

The lowest contents of the trypsin inhibitor were found when the plant was 12-months old for the Mocotó cv., followed by the IAC 289-70 cultivar. The higher contents of trypsin inhibitor in the 17-month old plants (starch accumulation phase) and the lower ones in the 12-month old plants (leaf development phase) are opposite to the results found by CORRÊA

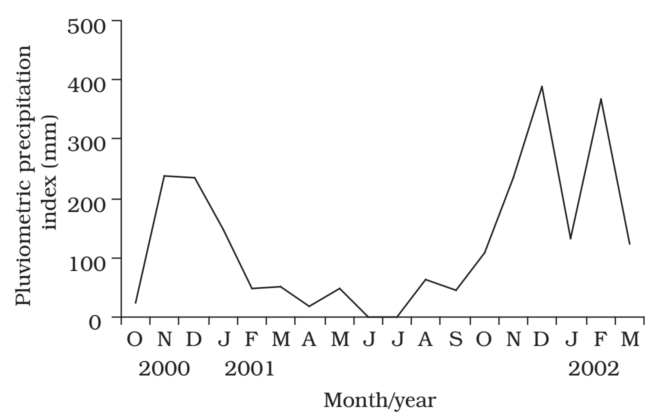

Figure 1. Pluviometric precipitation index from the planting time (October/2000) until the last collection of the leaves (March/2002) when the plant was 17-months old

Table 2. Average contents of polyphenol, trypsin inhibitor, saponin and the hemagglutinative activity in cassava leaf meal at three ages of the plant.

Cultivars	Polyphenol (mg.g^{-1} DM)[1]			Trypsin inhibitor (ITU[2].mg^{-1} DM)		
	12 mo	15 mo	17 mo	12 mo	15 mo	17 mo
Ouro do Vale	61.49[bA]	52.29[cC]	92.31[aB]	2.75[aA2]	1.88[bC]	2.80[aBC]
Maracanã	43.37[cC]	75.31[bA]	106.43[aA]	1.09[bC]	2.54[aB]	2.46[aD]
MANT.IAC	48.58[cB]	60.51[bB]	95.78[aB]	1.48[cB]	1.98[bC]	2.61[aCD]
IAC 289-70	47.33[cBC]	59.69[bB]	71.15[aD]	0.86[cD]	2.43[bB]	2.95[aB]
Mocotó	44.13[cBC]	78.86[bA]	79.88[aC]	0.57[bE]	3.13[aA]	3.28[aA]
Cultivars	Saponin (g.100 g^{-1} DM)			Hemagglutinin[3]		
Ouro do Vale	1.74[cD]	2.48[bD]	3.62[aC]	NDE[4]	ND[5]	NDE
Maracanã	2.28[cC]	3.20[bC]	4.43[aA]	1	NDE	NDE
MANT.IAC	2.95[cB]	3.35[bC]	3.61[aC]	0	NDE	ND
IAC 289-70	3.13[cB]	4.33[aB]	4.07[bB]	1	NDE	ND
Mocotó	4.41[bA]	4.73[aA]	4.38[bA]	0	NDE	ND

[1]Averages followed the by same small letters in the lines and capital letters in the columns do not differ from each others (Tukey test p≤ 0.05); [2]ITU = inhibited trypsin units; [3]The result points out the highest extract dilution that still produced a visible agglutination in blood (type A RH+). The number expresses the basis-2 exponent, concerning to dilution; [4]NDE = non-diluted extract; and [5]ND = non-detected.

et al.[14], who also investigated the influence of the plant age on the contents of the trypsin inhibitor in CLM from the Baiana cv., besides reporting the contents of 3.79 $ITU.mg^{-1}$ DM at the starch accumulation phase and 11.14 $ITU.mg^{-1}$ DM at the leaf development phase. The level differences of this antinutrient may be due to the cultivar itself.

An increase in saponin contents was observed with the maturity of the plant for most cultivars, except for IAC 289-70 cv. from which the levels in the 17-month old plant were lower than those at 15 months. The Ouro do Vale cv., at 12 mo, had the lowest saponin contents among all cultivars, as well as at any age of the plants. The saponin levels ranged from 1.74 to 4.73 g.100 g^{-1} DM, independent from both the cultivar and age of the plant. However, ONWUKA[35] analyzed the total leaves of four cassava clones dried in an oven (50 to 60 °C) and observed a variation from 0.18 to 0.25 g.100 g^{-1} for the equivalents of saponin, and quantified it by the method based on the erithrocyte hemolysis. In the present work, the colorimetric method was adopted, by dosing the steroidal sapogenins. These differences observed in the saponin contents are probably due to the different methodologies used in their quantification, as well as the influence of the cultivar, plant ages, and leaf maturity. The saponin contents in CLM are within the range described for soy beans seeds: 0.07 to 5.1 g.100 g^{-1} DM[15,16,23,44]. However, these levels are lower to the ones observed in alfalfa (5.6 g.100 g^{-1} DM) and beet leaves (5.8 g.100 g^{-1} DM)[15].

The hemagglutinative activity decreased with the maturity of the plant, but this tendency was not found in the cultivars Ouro do Vale and Maracanã FERNANDEZ et al.[17] observed the agglutination of erythrocyte solutions in crude extracts of the black, white and red common beans until dilution 10^4 and 10^6; therefore, CLM showed much lower values (from ND at 2^1). However, the toxicity is quite variable among the vegetative species. The ricin, for example, which is an hemagglutinin of the ricinus, is very toxic (LD_{50} of 0.05 $mg.kg^{-1}$ human body weight), in comparison with the hemagglutinin of the soy beans (Glycine max (L.) Merrill), from which the toxicity is a thousand times minor[43], as well as with that of the tomato (*Lycopersicon esculentum*) which is not toxic[32]. Therefore, the toxicological tests with the hemagglutinin of CLM would be advisable.

4 Conclusions

Among the ages of the plant considered in this study, the 12 month old ones had the lower contents for most antinutrients (oxalate, cyanide, polyfphenols, trypsin inhibitor and saponin). When the plant was 17 months old, however, CLM presented the lowest values for the nitrate content and agglutinative activity. The IAC 289-70 cv. presented the lowest levels of nitrate, cyanide, polyphnols and trypsin inhibitor.

Therefore, the IAC 289-70 cv. 12 month old is the most appropriate for the preparation of CLM, targeted to its use in the human feeding. Though, the others analyzed cultivars at TAP might be used in the preparation of the "multimistura" or consumed at amounts that do not cause toxicity.

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