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Effect of two agro-pedo-climatic zones, drying methods and pelleting processes on chemical composition of *Manihot esculenta* (sp.), *Leucaena leucocephala* and *Cajanus cajan*

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In tropical and subtropical countries, livestock productivity may be affected by the availability of food resources and the high prevalence of gastrointestinal pathogenic nematodes. The classical method of control using anthelmintic drugs is becoming decreasingly efficient because of a generalised resistance of the gastrointestinal nematodes suppress (GIN) to most of the drugs. In small farms, protein-rich biomasses with significant amounts of condensed tannins (CT), which are known to have anthelminbtic properties, might be good candidates to produce nutraceuticals. This experiment was conducted to determine the feasibility of producing nutraceutical pellets from *Manihot esculenta* sp., *Cajanus cajan* and *Leucaena leucocephala*, considering the influence of agro-pedo-climatic conditions plant species and technological factors, such as drying and pelleting. The samples were harvested in two different agro-pedo-climatic zones and sundried under shelter (at 25 to 35°C) or in a ventilated oven (45°C) before pelleting. Chemical analysis on crude protein and condensed tannins were conducted. The chemical composition of the plants did not vary significantly with agro-pedo-climatic conditions. Sun-drying and oven-drying decreased the CT content of the plants. No effect of pelleting was recorded on crude protein and CT contents, except for *C. cajan*, for which a small decrease in CT content was observed. Protein-rich foliage types with CT contents above 50 g/kg of dry matter are potentially good candidates to produce nutraceutical pellets if they are dried using mild drying conditions, like sun-drying under shelter.

Key words: Condensed tannins, nutraceuticals, drying, pelleting processes.

INTRODUCTION

Livestock productivity can be affected by gastrointestinal-induced pathologies that cause almost 45% mortality in sheep and goats before weaning. The classical method of control using anthelmintic drugs is becoming

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decreasingly efficient because of a generalised resistance of the gastrointestinal nematodes (GIN) to most of the drugs, especially in tropical areas (Mahieu, 2014). Many plants from tropical areas could be used as sources of nutraceuticals due to their composition of primary and secondary metabolites, and thus constitute part of an alternative to the use of anthelmintic drugs within integrated pest management systems against GIN (Cei et al., 2018; Santos et al., 2019).

Condensed tannins (CT) are of particular interest because they exert direct and indirect actions on pathogens (Hoste et al., 2012). These polyphenolic compounds could reduce the worm burden by impacting different steps of the development cycle of the nematode, as they have well-known actions on egg hatching rate, larval exsheathment and female fecundity (Hoste et al., 2012; Waghorn, 2008). Condensed tannins could also impact on the nutritional balance for the animals, given their influence on the quantity and the profile of available amino acids. By aiding to increase the bypass of dietary proteins in the intestine, condensed tannins protect proteins from degradation in the rumen (ruminal escape), causing increased lactation, wool growth and live weight gain, without changing voluntary feed intake (Piluzza et al., 2014).

Condensed tannin activities are known to depend on their concentration and nature (size, structure and profile) in the plant (Mueller-Harvey et al., 2019; Waghorn, 2008). Previous analysis shows that CT concentrations between 20 and 50 g/kg of the dry matter (DM) of the plant generate nutraceutical properties in the plant (Piluzza et al., 2014).

The CT composition depends on the plant species (Mueller-Harvey et al., 2019), plant growth stage and organs (Piluzza et al., 2014), as well as on the harvesting area, soil composition (Barry and Forss, 1983), humidity rate and weather conditions (Lees et al., 1994; Frutos et al., 2002). Moreover, the availability of a simple technology, like pelleting, suitable for the farmers and respectful of plant properties can improve the use of CT to manage GIN infections (Gaudin et al., 2016).

However, in order to produce plant pellets with an effective CT content against GIN infection, the sensitivity of CT to temperature has to be considered, as well as the variability of CT concentration in plants.

The global aim of this study was to assess the feasibility of pelleting condensed tannins-rich plants for their use as nutraceuticals at the farm level. For this purpose, experiments were undertaken to investigate the impact of natural conditions (soil, temperature and humidity) on the chemical composition, as well the influence of practices for preservation (drying and pelleting processes), on the CT content of four tropical plants.

MATERIALS AND METHODS

Foliage sampling: Collection and preparation

Four distinct types of plant foliage containing CT were chosen: *M. esculenta* sp.1; *M. esculenta* sp.2; *C. cajan* and *L. leucocephala*. *M. esculenta* sp.1 has lower levels of cyanhydric acid in its leaves and tubers compared to *M. esculenta* sp.2. The samples were harvested from two zones: (i) Grande–Terre that is characterised by a vertisol soil and humid tropical climate with a long dry season (3 to 5 months), 83% humidity and a mean temperature of 25°C; (ii) Basse–Terre that is characterised by a ferralitic soil and humid tropical climate with a short dry season (less than 2 months), 88% humidity and a mean temperature of 25°C. For each zone, the sampling was done from three sites, during the middle and the end of the dry season. Thirty to forty kilograms of stems of *L. leucocephala*, *C. cajan* and *M. esculenta*, aged 6, 8 and 12 months, respectively, were harvested.

These samples were mixed by site and then divided into three sub-samples that were dried under different conditions: Freeze-dried; sun-dried under shelter (at 25 to 35 °C); dried at 45°C for 2 days in a ventilated oven. These three sub-samples were used for chemical analysis, in triplicate. The freeze-dried and the sun-dried samples were pelleted without additives in a GR 150 E system (Oligotechnology, Wissembourg, France).

Chemical analysis and analytical procedures

The DM content was determined using a forced-air oven at 60°C until constant weight is achieved.

Foliage samples were milled through a 1 mm screen (Reich hammer mill, Haan, Germany) prior to analysis. Organic matter (OM) and nitrogen (N) were analysed according to the AOAC methods 923.3 and 992.15, respectively (AOAC, 1990). Crude protein (CP) content was estimated as N × 6.25. Cell wall components (neutral detergent fibre [NDF], acid detergent fibre [ADF] and acid detergent lignin [ADL]) were determined as described by Van Soest et al., (1991). CT content was determined on freeze-dried, sun-dried under shelter and 45°C ventilated-oven samples, respectively, using the vanillin-H_2SO_4 method reported by Laurent (1975). For improved accuracy, the CT concentration of the plant was determined using the CT extract of each plant as a standard for the individual calibration curves. The CT were extracted using a 70% (v/v) aqueous acetone solution (Giner-Chavez et al., 1997) and then isolated with Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO). Only N and CT were measured in the pellets. Each analysis was done in triplicate, and the means were calculated.

Statistical analysis

Statistical analysis was performed using the mixed general linear model procedure in SAS 9.2 (2008). The global model used to analyse the soil, the foliage and the drying process effects was:

$$Y_{ijkl} = m + F_i + Z_j + D_k + (F \times Z)_{ij} + (F \times D)_{ik} + S_l + e_{ijkl},$$

where m is the mean, F_i is the foliage fixed effect (i=1,2,3,4), Z_j is the zone fixed effect (j=1,2), D_k is the drying fixed effect (k=1,2,3), $(F \times Z)_{ij}$ is the interaction between the foliage and the zone effects, $(F \times D)_{ik}$ is the interaction between the foliage and the drying effects, S is a random effect associated with harvesting site and e_{ijkl} is the residual term.

The global model to analyse the pelleting process effect was:

$$Y_{ijk} = m + F_i + P_j + (F \times P)_{ij} + S_k + e_{ijk},$$

where m is the mean, F_i is the foliage fixed effect (i=1, 2, 3, 4), P_j is the processing fixed effect (j=1, 2, 3), $(F \times P)_{ij}$ is the interaction between the foliage and the processing effects, S is a random effect associated with harvesting site, and e_{ij} is the residual term.

Table 1. Chemical composition of *Manihot esculenta, Leucaena leucocephala* and *Cajanus cajan* foliage harvested from two different agro-pedoclimatic zones in Guadeloupe, France.

Item	Plant species				SEM	*p*-value	
	M. esculenta sp.1	*M. esculenta* sp.2	*L. leucocephala*	*C. cajan*		Agro-pedo-climatic zone[1)]	Zone × species
OM (g/kg DM)	894[a]	904[b]	910[bc]	922[c]	0.92	0.4991	0.0001
NDF (g/kg DM)	445[a]	422[ab]	378[b]	511[c]	2.51	0.4597	0.1157
ADF (g/kg DM)	247[a]	263[b]	189[c]	356[d]	1.21	0.4043	0.0001
ADL (g/kg DM)	109[a]	116[a]	91[c]	189[d]	0.73	0.4335	0.0003
CP (g/kg DM)	189[a]	189[a]	262[b]	217[c]	1.33	0.4337	0.0001
Ash (g/kg DM)	106[a]	96[b]	90[bc]	78[c]	0.92	0.4991	0.0001
CT (g/kg DM)	73[a]	60[b]	157[c]	170[c]	1.08	0.9546	0.0001

M. esculenta sp.1 with low cyanhydric acid content. *M. esculenta* sp.2 with high cyanhydric acid content.[1)] Vertisol and long dry season *vs*. ferralitic soil and short dry season. SEM, standard error of the mean; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; CP, crude protein; CT, condensed tannins. [a–d] Means within a row with different superscript letters differ significantly (p<0.05).

The values were expressed as least square means and standard error of the mean. Statistical differences were declared significant at p<0.05.

RESULTS

The harvested *C. cajan* and *L. leucocephala* seemed relatively homogeneous. Conversely, *M. esculenta* sp.1 and sp.2 constituted a more heterogeneous population, based on the size, the colour and the shape of the leaves, and the length and the colour of the petioles.

The chemical composition of the samples collected in this study is presented in Table 1. The comparisons of CP contents indicated significant differences between the plants (p<0.05), except for the two *Manihot* sp. (p=0.9502). For all foliage types, the CP content varied from 189 to 262 g/kg DM. Plants were also different based on the different components in the plant cell walls (NDF, ADF and ADL), excluding the two *Manihot* sp, for which the concentrations of NDF and ADL were similar. Regarding ash, no significant differences were observed between *L. leucocephala* and *M. esculenta* sp.2, and between *L. leucocephala* and *C. cajan. Manihot esculenta* sp.1 was significantly different from the other plants. CT concentrations were significantly different between the foliage types, except for *L. leucocephala* and *C. cajan*, which had similar concentrations with each other.

No significant effect of harvesting zone was registered on plant composition. However, interactions were observed between plants and harvesting zones. Although the differences were not significant, the plants harvested from Basse–Terre had NDF contents higher than those harvested from Grande–Terre. Similar observations were made for ADF, but the difference tended to be significant only for *C. cajan* (p=0.08). CP differences were not significant between harvesting zones for *L. leucocephala* and *C. cajan*, whereas, CP was significantly higher in *M. esculenta* sp.2 harvested from Basse–Terre (p=0.008), and higher, but not significantly, in *M. esculenta* sp.1 harvested from Basse–Terre (p=0.18). Although the differences were not significant, CT content was higher for the two *Manihot* sp. and *L. leucocephala* from Basse-Terre. In contrast, *C. cajan* CT levels were significantly higher in the plants harvested from Grande-Terre than Basse-Terre (p=0.0377).

The drying process significantly impacted on the CT content of the plants (Table 2). Sun-drying and oven-drying reduced the CT concentrations in plants. The most significant losses were observed with oven-drying at 45°C.

There was no significant influence of the pelleting operation on the N contents irrespective of the foliage and drying process considered (Table 3).

Pelleting noticeably affected the CT contents of *C. cajan*, only. The entire process (sun-drying then pelleting) significantly altered the CT contents of *M. esculenta* sp.2 and *C. cajan*. Comparing the sun-dried and pelleted plant to the freeze-dried plant, abnormally high CT contents were reported for *M. esculenta* sp.2, leading to a significant effect of the sun-drying and pelleting process.

DISCUSSION

General considerations

In this study, soil and rainfall were the parameters that had the most variation between the two areas. Temperature and humidity were quite the same. Globally, the chemical composition of leaves concurs with values reported in the literature (Table 4). The CP and CT values reported in literature vary in the ranges 168 to377 and 4 to 92 g/kg DM for *M. esculenta*; 153 to 403 and 9 to 181 g/kg DM for *L. leucocephala*; and 185.6 to

Table 2. Effect of drying technology on condensed tannin content in *M. esculenta, L. leucocephala* and *C. cajan* foliage.

Plant species	Condensed tannin content (g/kg DM)			SEM
	Freeze-dried	Sun-dried	Oven-dried	
Manihot esculenta sp.1	68.3[a]	40.8[b]	37.5[b]	0.871
Manihot esculenta sp.2	64.6[a]	26.4[b]	33.8[b]	0.858
Leucaena leucocephala	154.8[a]	135.6[b]	58.7[c]	0.831
Cajanus cajan	172.9[a]	152.8[b]	51.4[c]	0.926

DM, Dry matter; SEM, standard error of the mean. *M. esculenta* sp.1 with low cyanhydric acid content. *M. esculenta* sp.2 with high cyanhydric acid content. [a–c] Means within a row with different superscript letters differ significantly (p<0.05).

Table 3. Effect of pelleting technology on nitrogen and condensed tannins contents in *M. esculenta sp., L. leucocephala* and *C. cajan* foliage.

Species	Treatment			SEM
	Freeze-dried	Freeze-dried and pelleted	Sun-dried and pelleted	
Nitrogen (g/kg DM)				
Manihot esculenta sp.1	196.2	185.5	187.2	1.019
Manihot esculenta sp.2	194.8	176.2	187.2	0.974
Leucaena leucocephala	268.4	275.5	270.9	1.019
Cajanus cajan	228.2	221.5	225.5	1.096
Condensed tannins (g/kg DM)				
Manihot esculenta sp.1	66.3	59.9	57.5	0.7381
Manihot esculenta sp.2	93.1[a]	79.7[a]	19.2[b]	0.705
Leucaena leucocephala	138.8	156.9	137.9	0.7381
Cajanus cajan	172.4[a]	148.8[b]	129.1[b]	0.7934

M. esculenta sp.1 with low cyanhydric acid content. *M. esculenta* sp.2 with high cyanhydric acid content. [a–c] Means within a row with different superscript letters differ significantly (p<0.05).

236 and 47 to 77.1 g/kg DM for *C. cajan*, respectively. Considering the accuracy of the method for CP determination, it can be hypothesise that the results reported for these components are mainly due to natural variations in the plants. On the contrary, because of variations in methods, procedures and standards used for the analysis of CT (Frutos et al., 2002), it can be surmised that a large part of the variation reported in the literature is linked to those factors.

The high CP contents in the three distinct types of foliage evaluated, confirm their potential as feed resources. However, the digestibility and the amino acid profiles have to be taken into account to determine their nutritive value. The CP content of *L. leucocephala*, being the highest one, favours it as a good potential candidate in the development of a nutraceutical. *C. cajan* had the highest lignin content, which depresses digestibility. This plant cell wall component must be kept in mind when discussing the quality of foliage as a nutraceutical. Both *Manihot* sp. were similar in chemical composition, and so their method use can be the same for both species.

Effect of agro-pedo-climatic conditions on chemical composition

In the present study, significant effect of the agro-pedo-climatic conditions on the primary compounds (CP, cell wall) was not observed. This result can be explained by the low temperature and humidity variations and the absence of nutrient deficiencies in the soil of the two harvesting zones. This outcome is a classic result because, to the researchers' knowledge, no author has reported any significant effect under similar conditions. Even under adverse conditions, effects on primary components were low (Minson, 1990). Similarly, the effect of temperature only appears for large ranges of variation (Minson, 1990).

A variable effect of the agro-pedo-climatic conditions on the CT content, depending on the plant; hence, the

Table 4. Crude protein and condensed tannin contents in *C. cajan*, *L. leucocephala* and *M. esculenta* reported in the literature.

Species	CP (g/kg DM)	CT (g/kg DM)	Method for CT analysis	Reference
	236.0	-		Journal of Agricultural Science Research. 2016, 5(2):035-9.
	206.1	77.1	Vanillin–HCl–methanol; Price et al. (1978)	Journal of Biology, Agriculture and Healthcare. 2016, 6
	199.8	-		Journal of Animal Science. 2012, 41(3):717-25
C. cajan	199.8	-		Journal of Animal Science. 2000, 29(3):871-9.
	193.8	-		Tropical Grasslands. 1995, 29(4):263-9.
	185.6	-		Journal of Animal Feed Science and Technology. 1994;46:343.
	-	47.0	Butanol–HCl; Bate-Smith (1975) and Porter et al. (1986)	Journal of Range Management. 1994, 47(5):398-404.
Mean	**203.5**	**62.1**		
	403.0	181.0	Vanillin-HCl; Butler (1982)	Agroforestry Systems. 2003, 59(3):231-41
	306.0	18.0	Butanol-HCl–iron; Makkar (2003)	Animal Feed Science and Technology. 2011, 163(2-4):231-43.
	268.0	16.0	Butanol-HCl–ferric ammonium sulphate; Porter et al. (1986)	Asian-Australasian Journal of Animal Sciences. 2012, 25(10):1404-10.
	266.0	75;0	Vanillin-H_2SO_4; Laurent (1975)	Journal of Animal Physiology and Animal Nutrition. 2016, 100(6):1149-58.
	254.5	-		Brazilian Journal of Animal Science. 2012, 41(3):717-25.
	252.7	-		Journal of Animal Feed Science and Technology. 1994;46:343-8.
L. leucocephala	250.0	-		Animal Feed Science and Technology. 1998, 70(4):305-14.
	222.0	18.1	Vanillin–HCl–methanol; Price et al. (1978)	Global Journal of Animal Scientific Research. 2015;3(2):419-22.
	193.8	-		Tropical Grasslands. 1995, 29(4):263-9.
	193.0	9.0	Butanol–HCl; Porter et al. (1986)	Livestock research for rural development. 2008, 20 (11)
	153.0	12.7	Butanol–HCl; Makkar (2003)	Animal Feed Science and Technology. 2005, 119(3-4):345-61.
	-	134.0	Butanol–HCl; Makkar (1995)	Animal Feed Science and Technology. 2001, 91(1-2):95-106.
	-	129.5	Butanol–HCl; Terrill et al. (1992)	Journal of the Science of Food and Agriculture. 2004, 84(4):291-4
Mean	**251.1**	**65.9**		
	377.0	4.0	Butanol–HCl–iron; Makkar (2003)	Animal Feed Science and Technology. 2011, 163(2-4):231-43
	376.3	-		Brazilian Journal of Animal Science. 2012, 41(3):717-25.
	300.0	-		Animal Feed Science and Technology. 2013, 180(1-4):44-54.
	224.0	40.0	Vanillin–HCl; Nakamura et al. (2003)	Small Ruminant Research. 2010 Sep;93(1):10-8.
	208.0	92.0	Vanillin–H_2SO_4; Laurent (1975)	Journal of Animal Physiology and Animal Nutrition. 2016, 100(6):1149-58.
Manihot esculenta	208.0	21.6	Butanol–HCl; Porter et al. (1986)	Livestock Science. 2010, 129(1-3):24-30.
	200-300	43.0	-	Asian-Australasian Journal of Animal Sciences. 2003, 16(3):463-72.
	198.0	-		Animal Nutrition. 2016, 2(4):253-61.
	197.0	-		Asian-Australasian Journal of Animal Sciences. 2012, 25(12):1691-700.
	168.0	-		Livestock Science. 2010, 128(1-3):166-72.
	-	145.4	Butanol–HCl; Giner-Chavez et al. (1997)	Journal of the Science of Food and Agriculture. 1997, 74:359-68.
	-	81.6	Butanol–HCl; Terrill et al. (1992)	Journal of the Science of Food and Agriculture. 2004, 84(4):291-4.

Table 4. Contd.

	-	33.4	-	Journal of Agricultural and Food Chemistry. 1989, 37(3):712-6.
Mean	**250.6**	**45.1**		

DM, Dry matter; CP, Crude protein; CT, Condensed tannins.

absence of an overall effect was shown. The presence of a plant-dependent agro-pedo-climatic effect can be postulated. Some authors have shown a significant soil effect based on fertility and acidity. Low soil fertility or acid soil may lead to an increase in the rate of CT found in *Lotus* (Barry and Duncan, 1984; Kelman and Tanner, 1990). For some authors who have worked on contrasting agro-pedo-climatic conditions, the differences between CT concentrations varied from 70 to 400 g/kg DM, depending on the age of the plant (Muir et al., 2014), and from 50 to 20 g/kg DM, depending on the variety (Kelman and Tanner, 1990).

CT content may also vary with temperature and drought conditions. Lees et al. (1994) found an overall increase of 24% for big trefoil grown at 20 or 30°C. However, the effect of temperature could be age-dependent, as indicated in this same study. Indeed, the difference in temperature-related concentration decreased steadily from 36 to 9% for regrowth ages from 14 to 81 days.

When investigating *Lotus* grown on slightly acidic soils with a low-to-medium phosphorus content, Acuña et al. (2008) reported variations in CT due to water stress and temperature rather than to soil conditions. Similar results were found by Malisch et al. (2016) for sainfoin (*Onobrychis viciifolia* Scop.).

In this study, the effects of soil acidity and rainfall were mixed. Drought is more pronounced in basic soils than acid soils. It is not excluded that in addition to the problem of acidity, the issue of drought arises. The specific response of *C. cajan* on CT, compared with the two *Manihots* and *L. leucocephala* may be explained by its higher sensitivity to drought and not to acidity.

Effect of the plant species on the variation of chemical composition

In this study, the species was the main factor of variation for primary and secondary metabolites. According to the results, *C. cajan* and *L. leucocephala*, which are leguminous, are logically richer in N than the two *M. esculenta*. The NDF levels were relatively low compared with grasses because leaves analysed (Minson, 1990; Archimede et al., 2018).

As mentioned by Malisch et al. (2016), although the CT content of the plant is sensitive to external factors, the main factors of variation are of genetic and physiological origin.

In addition, the chemical characteristics of the plants are consistent with those found in the literature (Table 4). Moreover, intra-plant variations was not observe because the biomasses were harvested at similar ages and following the same procedures. The intra-plant variations reported were related to different varieties, or different ontogenetic stages (Malisch et al., 2016).

Effect of the drying and pelleting process on pellet composition

The process to produce the pellets was constituted by two operations: Drying and pelleting. CT are the components most sensitive to drying. The effect of different drying methods on CT: freeze-drying, sun-drying and oven-drying were first evaluated. In a second step, the type of process on the levels of CP and CT, which are important components from a nutraceutical perspective, was evaluated. In regards to drying, the results showed that oven-drying severely depreciated the CT content of the plants, whereas sun-drying had a more moderate effect when compared with the freeze-dried samples (control). This is a classical result. Indeed, depressive effects are reported only for temperatures above 55°C and for durations longer than 48 h.

Dzowela et al. (1995) and Hove et al., (2003), working on some tropical fodder shrubs, reported depressive effects for drying at 55 and 65 °C, respectively, for 48 h. Muetzel and Becker (2006) dried temperate plants at 60 °C for 2 h and specified that the effect of temperature could be plant-dependent.

Except for C. cajan, wherein the decrease was significant but relatively low (13%), pelleting did not have a depressive effect on the CP and CT contents compared with the corresponding values of freeze-dried non-pelleted samples and pellets obtained from freeze-dried plants. This behaviour can be explained by the technical parameters of the pelleting process used. During the pelleting, the temperature reaches 70 °C but the residence time of the plant particles in the apparatus is not sufficient to damage CP and CT. Indeed, since the pelleter has a capacity of 500 kg of forage per

hour, it was estimated that a plant particle stays in the pelleter for less than 1 min.

Conclusion

This study did not show any major effects of agro-pedo-climatic conditions on the variation of the chemical composition of the targeted plants for pelleting. This result can be explained by the absence of major stress to the plant. The main factor of variation was drying. Consequently, under a mild drying condition, like sun-drying under shelter, the main recommendation would be to select forages with CT content above 50 g/kg DM, to ensure post processing nutraceutical properties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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