Chemical composition and *in vitro* degradation of red and white mesquite *(Prosopis laevigata)* pods

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Abstract

The objective of this study was to compare the chemical composition and ruminal degradation of whole pod, exomesocarp, endocarp and seed fractions of red and white mesquite pods. The pods contained on average 220 g free sugars, 78 g crude protein, 21 g fat per kg dry matter (DM), and a potential DM degradation of 163 g/kg. Contaminant fungi (mostly *Aspegillus* spp.) count was low. Unsaturated fatty acids, mainly linoleic acid, were the predominant (~50%) fatty acids in whole pods and seeds. Sucrose was the largest free sugar proportion. The highest fibre content was found in the endocarp, the highest free sugar was found in the exomesocarp, and the highest crude protein content was found in the seeds. Tannins were more abundant in red pods (0.4 mg/100 g DM) than in white ones. Some differences in nutritional values were found between red and white pods and their components (exomesocarp, endocarp and seeds), although both have a potentially high nutritive value. Whole pods and the endocarp can be used by ruminants; seeds can be used by simple stomach animals; and the exomesocarp can be used in human nutrition because of its low glycaemic index properties.

Keywords: Degradation rate, fatty acids, sugar, tannins
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Introduction

Several species of mesquite (*Prosopis* spp*.)* are well distributed in and adapted to arid and semi-arid regions of the world. Approximately, 40 species (trees and shrubs) are native to North and South America (Pasiecznik *et al.,* 2001). However, they have been introduced and have adapted to dry lands of Asia, Africa and Australia because of their commercial value, contribution to land rehabilitation, and provision of fodder and firewood (Mwangi & Swallow, 2008; Mworia *et al*., 2011). Mesquite pods (referred to henceforth as fruits) vary in colour from white to red and dark brown. They have been used as human food (Felker *et al*., 2013) and their extracts have been extensively evaluated as a source of nutraceuticals (Bernardi *et al.*, 2010) and pharmaceuticals (Huisamen *et al*., 2013; Mollashahi *et al*., 2013). The fruits are also used as animal feed (de Jesus Pereira *et al*., 2013) which, depending on the colour, may have different effects in the rumen (Cabiddu *et al*., 2010) because of variation in content of phytochemical compounds (mainly polyphenols) (Parveen *et al*., 2010)). Incidents of invasion of mesquite species as a result of seed dispersal by livestock (Sawal *et al*., 2004), wildlife and water have been reported in north-east Ethiopia (Shiferaw *et al*., 2004), forest riverines of Kenya (Muturi *et al*., 2013), and savannas and grasslands of Argentina and southwestern USA (Golubov *et al*., 2001). The fruits are equipped with biological characteristics that foster their rapid invasion into new areas (Shiferaw *et al.,* 2004). For example, they produce many small hard seeds with attractive colours, and are capable of surviving passage through the digestive system of animals. The sweet mesocarp contains a mixture of seeds that can germinate quickly or remain dormant for a long time. When consumed unground, the number of seeds recovered from 1 kg faeces of cattle, warthogs, camels and goats were 2833, 2344, 1642 and 760, respectively (Shiferaw *et al*., 2004; Riet-Correa, 2011). Grinding the fruits helps to break the hard capsule surrounding the seed, improves the digestion of nutrients, and prevents seed

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dispersal. The fruit can be fragmented mechanically into the exomesocarp (13%) (by weight), endocarp (16%), and seeds. The exocarp is the external layer that covers the spongy medium layer, which is known as mesocarp or pulp, and is composed mainly of carbohydrates. The endocarp, or internal layer, is a hard stony case that protects the seeds. The mesocarp and the endocarp may obstruct the sieve during grinding, hindering proper crushing of the seeds, which is where most nutrients are found (Freyre *et al*., 2003). Most previous research compared the chemical composition of several species of mesquite (Astudillo *et al*., 2000; Batista *et al*., 2002; Freyre *et al*., 2003; González-Galán *et al*., 2008; Andrade-Montemayor *et al*., 2009), but none evaluated the effect of fruit colour on nutritional quality. Therefore, the objective of this study was to compare the chemical composition and ruminal degradation of intact fruit (referred to as whole pod), exomesocarp, endocarp and seeds of red and white mesquite varieties.

Material and Methods

Mesquite fruits were collected from six adult trees (~13 years old) located in the city of San Luis Potosí, México, in May and June 2011. Three trees produced white fruits and three red ones. Approximately 27 kg fruit per tree were collected. One portion (~20 kg) of whole white or red fruits was separated prior to analysis for dry matter (DM), ash, and crude protein (CP). The DM percentage was determined by loss of weight after drying 200 g for each sample at 55 ºC in an air-forced oven until constant weight (AOAC Official Method 930.15; AOAC, 2005). Then, each group of fruits was ground with a Wiley mill using a 1 mm sieve screen (Model 4; Arthur H. Thomas Co. Philadelphia, Pa., USA). Ash content was determined subsequently at 550 ºC for 5 hours in a muffle furnace (Method 942.05; AOAC 2005). The Kjeldahl method was used to determine nitrogen (N) (AOAC Official Method 976.05; AOAC, 2005), with the CP content calculated as N x 6.25. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were assayed according to procedures described by Van Soest *et al*. (1991) with modified ANKOM 200 fibre analyser apparatus (ANKOM Technology Corporation, Fairport, N.Y. USA). Neutral detergent fibre analysis was conducted using sodium sulphite and alpha amylase (heat stable). A second portion of the fruits (~40 kg) was used to obtain the following fractions: exomesocarp, endocarp and seed. First, the fruits were air-dried for 32 h under a soft shade to facilitate the grinding process by preventing the formation of lumps from water and sugars in the fruits. Dried fruits were ground through a Bear Cat mill with a 3 inch screen (Crary Industries Inc., West Fargo, N. Dak., USA). Then, ground fruits were separated through a 4.76 mm mesh (sieve No.8), where a mixture (outer cuticle, pulp and seed fruits) passed through the sieve and the internal layer or endocarp was separately. The exocarp and mesocarp size were similar and were difficult to separate. Thus, seeds were obtained by manual separation. Then, samples of the exomesocarp, endocarp and seeds were ground again, but with a Wiley mill using a 1 mm sieve screen to determine DM, CP, ash, NDF and ADF, as described above.

To quantify long-chain fatty acids, methyl esters from fatty acids (FAMEs) were obtained by alkaline methanolysis, according to Gómez-Brandon *et al*. (2008). Total fatty acids were extracted from 200 mg samples with 12 mL of chloroform methanol, 2 : 1 (v/v). Total lipid extracts were obtained with a 0.2 M KOH methanol solution and toluene-methanol 1 : 1 by vortexing for 60 seconds and re-extracted twice. FAMEs were extracted with a 4 : 1 hexane-chloroform solution and water, centrifuged at 4545 x *g* for 5 minutes. The upper phase was transferred to another test tube and the lower phase was re-extracted twice with the same solutions, evaporated under a N_2 flow to a final volume of 0.5 mL. Ten microlitres of methyl heptadecanoate (C17:0 at 0.26 mg/mL, Sigma-Aldrich) were added as chromatographic standard to 100 µL samples of each extract. Composition analysis was performed in a gas chromatograph with a flame ionization detector (6890N, Agilent Technologies Systems, USA) and a capillary column HP-INOWax 30 m x 0.32 mm x 0.25 µm with programmed temperature (150 ºC) for 1 min, increased by 5 ºC/min to reach 230 ºC for 13 minutes), using helium as carrier gas and a constant flow rate of 1.5 mL/min. The standard used to identify the fatty acids was Fame Mix C14-C22 (Supelco, Bellefonte PA, USA).

Sugar profiles were obtained from a solid-liquid extraction procedure. The crude lipid fraction was removed from a 0.5 g of sample that was dried and finely ground (1 mm) using petroleum ether, with a final volume of 12 mL, centrifuged 4545 x *g* for 10 min (HN-SII Centrifuge International Equipment Company, KY,USA). Petroleum ether was aspirated and discarded twice without siphoning off solid material. Residual petroleum ether was then evaporated with a gentle stream of N_2, according by the International AOAC (982.14; AOAC, 2006). Dried and defatted powder were spiked with an internal standard, lactose (6 mg/mL), and extracted with 10 mL 80% aqueous ethanol at 70 ºC for 30 min. The resulting suspension was centrifuged at 11363 x *g* for 15 min. The supernatant was concentrated at 40 ºC under reduced vacuum, until total ethanol removal, and then diluted in water (Mili-Q, TGI Pure Water Systems, USA) to a final volume of 10 mL, according by Barreira *et al.* (2010). The carbohydrate profile was determined by high-performance liquid chromatography with a high refraction detector (HPLC-IR) in an 1100 series chromatographer (Agilent Technologies Systems, Santa Clara, Calif.) with a Zorbax Carbohydrate column (4.6 mm ID x 150 mm (5

µm) at 30 ºC. The mobile phase involved acetonitrile/deionized water, 75 : 25 (v/v), at a flow rate of 1.4 mL/min, and the injection volume was 20 µL. The results were expressed in g/100 g dried weight, calculated by internal standard normalization of the chromatographic peak area. Sugar identification was made by comparing the relative retention times of sample peaks with standards. Reference standards were from Sigma (St Louis, Mo, USA).

Tannin content was determined according to the ISO standard (ISO 9648:1988). A solution of dimethylformamide at 75% (10 mL) was mixed with ground samples (500 mg) and shaken (Speci-Mix Thermolyne, Barnstead International, Boston, Mass, USA) for 60 min for tannin extraction, before centrifugation at 3000 x *g* for 10 min. Supernatant (0.5 mL) was transferred in a test tube containing a mixture of ammonia (0.5 mL) and water (3 mL). In another test tube, 0.5 mL of supernatant was mixed with a 3.5 mL mixture of 0.5 mL ferric ammonium citrate, 0.5 mL ammonium hydroxide and 2.5 mL water. The tube was shaken for 60 sec, and placed to rest for 10 min before an aliquot was transferred into measuring cells to determine absorbance at 525 nm, with an UV-visible spectrophotometer (HP 8453; Agilent Technologies). Tannic content was determined after preparing a calibration curve (0.05, 0.1, 0.2, 0.3, 0.4 mg/mL) with tannic acid.

For contaminant fungi, the horizontal method (PNT-Al-006) based on the norm XF V08-059 was used to quantify yeast and moulds, according to Allaert Vandevenne & Escolá Ribes (2002). One g sample was suspended in 9 mL sterile water, mixed at 25 ºC and diluted. One mL of each dilution (10-1–10-5) was placed in a Petri dish with Sabouraud dextrose agar (SDA) supplemented with 0.40 mg/L gentamicin and 400 mg/L chloramphenicol. The dishes were mixed gently and incubated at 25 ºC for five days. Fungal species were isolated by re-plating, for an additional 72 h incubation, and identified by direct microscopy with cotton lacto-phenol blue staining.

In vitro degradation of DM was carried out according to the procedure of Tilley & Terry (1963) and degradation kinetics of DM was conducted as described here. Ruminal fluid was collected from two ruminally cannulated lactating cows that had free access to water and a 70 : 30 forage : concentrate diet offered in two equal portions at 08:00 and 16:00. The forage was a mixture of maize silage and lucerne hay (70 : 30). The concentrate mixture contained wheat middling, corn maize, soybean meal and mesquite fruits (20 : 40 : 20 : 20). For each of seven replications, three tubes of whole pods, endocarp, exomesocarp and seeds from white and red fruits were incubated for 0, 3, 6, 12, 24, 48 and 72 h. *In vitro* ruminal kinetics of DM were calculated using the Gompertz model (2) as outlined in Susmel *et al.* (1999):

$$deg_{(t)} = (a + b) \exp[(-C) \exp(-Dt)]$$

where: deg is the DM degraded (g/kg) at time *t*; *a* is the immediately soluble DM fraction (g/kg), *b* is the insoluble, but potentially degradable fraction (g/kg) over time (t, hr); a+b is the total substrate potentially degradable (soluble and degradable); *C* is the fractional degradation rate of *a*+*b*; and *D is* a parameter to consider the microbial biomass. According to the Gompertz model, fractional rate of degradation varies as a function of time, and an average value (i.e. a constant value comparable with the exponential rate of degradation) can be derived as c = D/C. For each incubation time, the residual DM in each tube was averaged before fitting the data to a nonlinear regression model using the NLIN option of SAS (2002).

Analyses for chemical composition, FAMEs, sugar profile, tannins and fungi contaminant were conducted in triplicate. Analysis of variance of these dependent variables was conducted with SAS (2002), using the GLM procedure and the Tukey test to separate the treatment means. *In vitro* degradation parameters (*a, a*+*b*, and *c*) were subjected to one-way variance analysis using a mixed model (SAS, 2002), that included treatment as a fixed effect and replication as a random effect. Differences among treatments were declared at $P < 0.05$.

Results and Discussion

Mesquite fruits had an average content of 856 g DM/kg, 329 g NDF/kg DM, 252 g ADF/kg DM, 260 g sugar/kg DM, 78 g CP/kg DM, 41 g ash/kg DM and 21 g fat/kg DM, and 0.25 mg tannins/100 g DM (Table 1). Differences in composition between the white and red whole pods are presented in Table 1. The fungi species identified were mostly from the *Aspergillus* genus (*A. nidulans*, 88 FCU/100 CFU; *A. fumigatus*, 4 FCU/100 CFU; *A. niger*, 3 FCU/100 CFU; *A. flavus* - *A. terreus*, 1 FCU/100 CFU), which is the most common fungus genus found in animal feeds (Azarakhsh *et al*., 2011). Other fungi identified in fruits in this study included *Fusarium* spp. and *Mucor* spp. The counts for all the fungi were considered low according to Mexican regulations (NOM-111-SSA1-1994). Evidence from previous work (Boyd & Cotty, 2001; Canafoglia *et al*., 2007) indicated that fruits are susceptible to damage by insects when growing in the vicinity of crop fields, especially bruchids such as *Algarobius johnsoni*, *A. atratus* and *A. johnsoni* (Kingsolver *et al.*, 1986). Fruits damaged by insects may become reservoirs of fungi, some with aflatogenic capacity. However, the levels of aflatoxins found here were not higher than commonly observed in conventional human foods (Kaaya & Warren, 2005).

Table 1 Chemical composition of whole pods, exomesocarp, endocarp and seed of mesquite

Component	Whole pod			Exomesocarp			Endocarp			Seed		
	White	Red	SEM	White	Red	SEM	White	Red	SEM	White	Red	SEM
Dry matter (DM), g/kg	855	858	5.9	917[b]	931[a]	5.4	931	932	4.4	946	950	6.3
NDF, g/kg DM	307[b]	352[a]	3.3	387[b]	422[a]	4.4	615[b]	688[a]	4.1	167[b]	182[a]	3.9
ADF, g/kg DM	237[b]	272[a]	2.7	244[b]	325[a]	2.5	527[b]	553[a]	2.6.	111[b]	127[a]	3.7
Total sugar, g/kg DM	198[b]	242[a]	3.9	215[b]	305[a]	3.9	113	110	3.9	11[a]	8[b]	0.6
CP, g/kg DM	77[b]	79[a]	0.7	57[b]	61[a]	1.7	22[b]	29[a]	1.1	259[b]	285[a]	2.9
Ash, g/kg DM	39[b]	43[a]	0.2	33[b]	37[a]	0.4	53[b]	59[a]	0.5	36[a]	35[b]	0.3
Fat, g/kg DM	27[a]	15[b]	0.7	20	19	1.1	9	10	0.8	38[a]	26[b]	1.1
Tannins, mg/100 g DM	0.1[b]	0.4[a]	0.1	0.1	0.1	0.1	0.2[a]	0.1[b]	0.02	0.4	0.4	0.02
Fungi, $\log_{10}$ CFU/g	2.7	2.8	0.1	3.4[a]	1.0[b]	0.1	2.7[a]	0.5[b]	0.05	1.3[a]	0.5[b]	0.04

DM: dry matter; NDF: neutral detergent fibre; ADF: acid detergent fibre; CP: crude protein; SEM: standard error of mean.
[ab] Within rows white and red means for whole pod, exomesocarp, endocarp and seed with different superscripts differ at P <0.05.

Table 2 Fatty acid profile and individual free sugar in whole pods, exomesocarp, endocarp and seeds of mesquite

	Whole			Exomesocarp			Endocarp			Seed		
	White	Red	SEM	White	Red	SEM	White	Red	SEM	White	Red	SEM
Fatty acid, g/100 fat												
C14:0	0.1[b]	0.2[a]	0.01	0.1	0.1	0.01	0.6[a]	0.1[b]	0.03	0.1	0.1	0.01
C16:0	21.1	18.6	1.10	18.5	19.1	1.93	20.3	18.8	0.81	15.9[a]	15.4[b]	0.49
C18:0	4.0	4.7	0.61	4.4	4.7	0.41	4.8[a]	4.4[b]	0.42	3.9	3.7	0.40
C18:1n9t	13.0	14.6	0.69	16.5[b]	18.1[a]	0.38	14.9	15.6	0.46	17.1[a]	16.2[b]	0.55
C18:1n9c	6.7[a]	5.1[b]	0.41	47.8	46.2	0.84	49.7	52.0	1.51	1.2	1.3	0.11
C18:2n6c	52.0	51.8	1.88	6.2	5.5	0.69	4.9[a]	3.7[b]	0.30	55.5[b]	57.9[a]	0.94
C18:3	1.6[b]	2.1[a]	0.05	4.5	4.1	0.67	2.2	2.2	0.03	2.7	2.1	0.29
C20:0	1.9[b]	2.6[a]	0.17	1.9	2.1	0.16	2.5[b]	2.9[a]	0.04	3.2	2.9	0.22
C22:0	0.1[b]	0.3[a]	0.02	0.1	0.1	0.01	0.1[b]	0.3[a]	0.06	0.4	0.4	0.01
Free sugar, g/100 g sugar												
Glucose	5.2[b]	7.2[a]	0.26	2.0	1.8	0.21	3.1	2.5	0.42	0.1[b]	0.2[a]	0.01
Fructose	11.3[b]	11.8[a]	0.17	12.0[a]	9.5[b]	1.00	12.9	14.4	0.87	18.4	19.1	1.34
Sucrose	83.5	81.0	1.42	86.0	88.7	1.10	84.0	83.1	1.23	81.5	80.7	1.35

[ab] White and red means for whole pod, exomesocarp, endocarp and seed with different superscripts differ at $P < 0.05$.

Table 3 *In vitro* degradation of dry matter in whole pods, exomesocarp, endocarp and seeds of mesquite

Gompertz	Whole pod			Exomesocarp			Endocarp			Seed		
parameter	White	Red	SEM	White	Red	SEM	White	Red	SEM	White	Red	SEM
a, g/kg DM	492	438	30.1	226[b]	435[a]	24.9	158	147	10.9	267	290	20.6
b, g/kg DM	189[a]	137[b]	10.3	282[b]	358[a]	26.7	392	428	29.1	389	431	23.5
a+*b*, g/kg DM	681[a]	575[b]	38.1	508[b]	793[a]	37.1	550	575	33.8	656	721	30.1
c, /h	0.032	0.023	0.006	0.022	0.020	0.004	0.029	0.021	0.004	0.027	0.023	0.004

DM: dry matter.
A: soluble fraction; *b*: potentially degradable fraction; *a*+*b*: total degradation; *c*: degradation rate.
[ab] White and red means for whole pod, exomesocarp, endocarp and seed with different superscripts differ at $P < 0.05$.

Nine fatty acids were identified (Table 2). Linoleic acid (C18:2n6c) was the predominant (~50 g/100 g fat) unsaturated fatty acid in whole pods and seeds, followed by oleic acid (C18:1n9c) and elaidic acid (C18:1n9t). Palmitic acid (C16:0) was the major component among the saturated acids. The fatty acid profile reported here was similar to that of conventional vegetable oils (NRC 2001), and was in agreement with the reports of Lamarque *et al*. (1994) and Freyre *et al*. (2003). Sucrose was the free sugar in largest proportion, followed by fructose and glucose. The sugar profile was similar to sugar beet (NRC, 2001) and in similar proportions to those reported by Marangoni & Alli (1988). The values were within the ranges reported by Sawal *et al.* (2004) who reviewed the chemical composition of several mesquite species.

The CP level (Table 1) was similar to conventional grains such as maize, wheat and sorghum. The mesquite fruits contained more fibre than conventional grains, but less than roughages and by-products such as maize stover, wheat straw, cotton seed and wheat bran. Mesquite fruits have been used to replace forages, by-products, conventional grains and commercial concentrates in experiments that did not consider their unique properties when formulating diets (e.g., Mahgoub *et al*., 2005a; b; Andrade-Montemayor *et al*., 2009; Koech *et al*., 2010; de Jesus Pereira *et al*., 2013). Future research, however, should consider these properties when comparing mesquite with conventional feedstuff.

Whole pods, exomesocarp and seed from white and red fruits had similar DM contents (Table 1). As expected, the highest fibre content was found in the endocarp, the highest concentration of free sugar was found in the exomesocarp, and the highest crude protein content was found in the seeds (Table 1). Whole pods, and the exomesocarp, endocarp and seed of the red fruits had higher (P <0.05) NDF, ADF and CP concentrations than those from white fruits, whereas the fat concentration was higher (P <0.05) in whole pods, white fruits and their seeds than those from red fruits. In contrast, whole pods, exomesocarp and endocarp of red fruits had a higher (P <0.05) ash content than the white fruits. Fatty acids (C14, C18, C18:2n6c, C20, C22) concentrations of red whole pod and endocarp were higher than in the white counterparts. In contrast, fatty acids (C16, C18:1n9t, C18:1n9c) concentrations of white whole pod and seed were higher than in the red counterparts. Free sugar profiles were higher (P <0.05) in red whole pod and seed than in the white counterparts.

Tannin level was highest (P <0.05) in the endocarp of white fruits, and was higher in red whole pods than the white whole pods. In the present study, tannin levels were lower than the tannic acid levels reported by González-Galán *et al*. (2008). Tannin level in mesquite fruits may not always be a limiting factor in its dietary inclusion level, as suggested by Mahgoub *et al*. (2004). Contaminant fungi counts were higher in the exomesocarp, endocarp and seed of white fruits compared with the counterpart fractions of red fruits. There are no previous studies that evaluated the effect of fruit colour on chemical composition in mesquite, but evidence with Ilama (*Annonia diversifolia* Safford) fruits indicated that colour influences the chemical composition owing to phytochemical compounds (Julian-Loaeza *et al*., 2011).

In whole pods the potentially degradable fraction (*b* fraction) and total degradation (*a* + *b* fraction) were higher (P <0.05) in white than in red fruits (Table 3). However, in the exomesocarp, the soluble fraction (*a* fraction), the potentially degradable fraction (*b* fraction) and total degradation (*a* + *b* fraction) were higher (P <0.05) in red fruits than in white fruits. There were no differences in degradation rates among the fruit fractions. The highest *in vitro* degradation parameters in exomesocarp could be related to the higher proportion of CP in red fruits compared to white fruits. Whole pods could be a potential raw material for the human food industry. However, they could be used in the production of bio-fuel, as suggested by González-Galán *et al*. (2008). Batista *et al*. (2002) found that digestible DM of fruits was high (approximately 680 g/kg DM), suggesting a high ruminal availability. The digestibility values were comparable with conventional cereal grains such as wheat (895 g/kg DM) and corn (899 g/kg DM) (Aye-Saldar *et al*., 2012).

Conclusion

Chemical composition and ruminal degradation of mesquite fruits used in this experiment were similar to several conventional animal feeds. Each fraction (exomesocarp, endocarp and seeds) has its distinct composition, which contributed uniquely to the overall nutritional value of the whole pod. Although differences were found between red and white varieties of mesquite, both have great potential for the development of local and sustainable feed production systems with low environmental impact and cost of production.

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