

## Nutrient composition and *in vitro* digestibility of Bambara groundnut (*Vigna subterranea*) haulm as affected by drying methods

Ogunbote O. B, Amisu A. A, Adeoye S. A, Ayantayo F. E, Ilubusi O. T, Adeniyi O. R, Abdul-azeez T. O, and Arigbede O. M.

Department of Pasture and Range Management, Federal University of Agriculture, Abeokuta, Ogun State.



Corresponding author: [ogunboteob@funaab.edu.ng](mailto:ogunboteob@funaab.edu.ng); +2348134777459

### Abstract

*Vigna subterranea* is an underutilized legume with limited knowledge about drying methods that can prevent its nutrient's degradation and available to ruminant. The experiment was carried out to investigate the effects of different drying methods on the nutrient composition and *in vitro* digestibility of Bambara groundnut haulm. The study was carried out at the Center for Agriculture and Sustainable Environment (CEDEASE) screen house and the legume was sown at a rate of 2 seeds per bucket. The legume was harvested at twelve weeks after sowing, pods were separated before the remaining haulm was measured and divided into three equal parts before it was dried under the three drying methods until constant weight and milled for nutrient composition and *in vitro* digestibility studies. Gas production parameters were recorded at 3, 6, 12, 15, 18, 21 and 24 hours of the incubation period. The drying methods had significantly different on the proximate parameters expect for ether extract. Air dried Bambara groundnut had the highest value (17.40%) of crude protein (CP) while Sun dried had the least value (16.33%) for CP, on the other hand, the fibre content of Bambara groundnut were not significantly ( $p>0.05$ ) different expect for cellulose where the sun dried legume had the highest content of cellulose (24.03%). There were significant differences ( $P<0.05$ ) in the incubation hours, sun dried sample also had the highest gas production at the end of the incubation period and was highest ( $P<0.05$ ) for short-chain fatty acid and organic matter digestibility also. It was concluded that air drying samples can be adopted for improved crude protein and improve IVDMD but reduces OMD and SCFA.

**Keywords:** Proximate composition, *in vitro* digestibility, Legumes, Drying methods

## Composition nutritionnelle et digestibilité *in vitro* des fanes de pois bambara (*Vigna subterranea*) selon les méthodes de séchage



### Résumé

*Vigna subterranea* est une légumineuse sous-utilisée, avec peu de connaissances sur les méthodes de séchage permettant d'éviter la dégradation de ses nutriments et de les rendre disponibles pour les ruminants. Cette étude a été menée pour évaluer les effets de différentes méthodes de séchage sur la composition nutritionnelle et la digestibilité *in vitro* des fanes de pois bambara. L'expérience a été réalisée dans la serre du Centre pour l'Agriculture et l'Environnement Durable (CEDEASE), où la légumineuse a été semée à raison de 2 graines par seau. La récolte a eu lieu douze semaines après le semis, les gousses ont été séparées, puis les fanes restantes ont été pesées et divisées en trois parties égales avant d'être séchées selon trois méthodes différentes jusqu'à poids constant, puis broyées pour l'analyse de la composition nutritionnelle et de la digestibilité *in vitro*. Les paramètres de production de gaz ont été enregistrés à 3, 6, 12, 15, 18, 21 et 24 heures d'incubation. Les méthodes de séchage ont eu un effet significatif sur les paramètres proximaux, à l'exception de l'extrait éthéré. Le pois bambara séché à l'air a présenté la teneur la plus élevée (17,40 %) en protéines brutes (PB), tandis que le séchage au soleil a donné la valeur la plus faible (16,33 %). En revanche, les teneurs en fibres n'ont pas varié significativement

( $p > 0,05$ ), sauf pour la cellulose, où le séchage au soleil a donné la teneur la plus élevée (24,03 %). Des différences significatives ( $P < 0,05$ ) ont été observées selon les heures d'incubation : l'échantillon séché au soleil a également produit le plus de gaz en fin d'incubation et a présenté les valeurs les plus élevées ( $P < 0,05$ ) pour les acides gras à chaîne courte (AGCC) et la digestibilité de la matière organique (DMO). En conclusion, le séchage à l'air peut être adopté pour améliorer la teneur en protéines brutes et la digestibilité *in vitro* de la matière sèche (DIVMS), mais il réduit la DMO et les AGCC.

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**Mots-clés : Composition proximale, Digestibilité *in vitro*, Légumineuses, Méthodes de séchage**

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## **Introduction**

Ruminant production is a major component of the livestock sector in Nigeria, they major source of food security serving a diverse function including cash income, savings, cash for fertilizer purchase and sociocultural functions (Solomon *et al.* 2013), however, one of the major factors facing livestock productivity is lack of feed, both in quality and quantity (Anele, *et al.*, 2011) as seasonal fluctuation hinders the small livestock holder to maximize their potentials in Nigeria and other parts of West African (Ayantunde *et al.* 2005; Anele *et al.* 2011). Ruminant animals rely on pasture for their nutrition than any other feed resources (Aderionola *et al.*, 2008) as this is the cheapest and the major component in their diets, lack or almost complete absence of forage conservation leads to huge depreciation in quality, as forages that grow abundantly as natural pastures during the rainy season are allowed to mature and dry out by the onset of the dry season, pasture tends to be more succulent, highly nutritious and more abundant in the rainy season (May - November) as opposed to the dry season (November - April) where they become fibrous, scarce and devoid of most essential nutrients such as protein, energy, minerals and vitamins which are required for increased rumen microbial fermentation that will results into production of volatile fatty acid and consequently performances of the host animal in the area of maintenance, production and reproduction (Ojo *et al.*, 2023). At this period, the performance of ruminant animals which is dependent on the native pasture is seriously impaired; the quality is associated with the fibrous and lignified nature of

the pasture which limits intake, digestibility and utilization (Markos *et al.*, 2006). In the tropics they are raised predominantly on grasses which are inherently poor in digestibility, nutritive value and unavailable in the off season (Babayemi, 2009). Mixture of grasses and legumes is a viable alternative to maintain and increase animal production (Hirai *et al.* 2015). Legume is easily established and fast growing to maturity over a short period of time. They perform to enhance human health (Tharanathan and Mahadevamma 2003.), support the production of farm animals (Nichols *et al.*, 2007), enrich soil conditions (Biederbeck, *et al.*, 2005), and mitigate the impact of greenhouse gases (Bayer *et al.*, 2016). By covering the soil surface, the vegetative portion of the crops protects the soil from wind and rain erosion. Legume is used by farmers for feeding ruminant because of its nutrient composition (Pen *et al.* 2013). Legume plants are known as green concentrate because they contain high protein and digestibility comparing grass (Heinritz, *et al.*, 2012). The drying process is the best method to keep the nutrients and bioactive compounds intact to their maximum level. Also, reduces the cost of the final product because the weight determines the transportation as well as storage charges. Furthermore, the shelf life of the product also increased because moisture is the key factor for microorganism growth (Mediani *et al.*, 2014). Dehydration of the legumes can be done by using various methods (Salarikia *et al.*, 2017 and Periche *et al.*, 2015). It is very important to draw attention to the effect of different drying techniques on the nutrition (Lin *et al.*, 2011). Gas production is one of *in vitro*

parameter that is very useful to predict digestibility value. Furthermore, *in vitro* gas production system can predict methane gas production *in vivo* (Babayemi, 2009). The *in vitro* method of feed evaluation is less expensive and less time consuming compared with *in vivo* methods. The *in vitro* gas production system helps to better quantifying the nutrient utilization and its accuracy in describing digestibility in animal has been validated in numerous experiments (Sallam, *et al.*, 2007).

### **Materials and Methods**

The planting was carried out at the Center for Agriculture and Sustainable Environment (CEDEASE) screen house at the College of Plant Science and Crop Production (COPLANT), while the chemical analysis was carried out at the laboratory of the Department of Pasture and Range Management, College of Animal Science and Livestock Production. Both sites are located at FUNAAB, Ogun state, Nigeria. The Seed was sourced from a reputable agriculture store within Abeokuta, and was sown at a rate of 2 seeds per bucket. The legume was harvested at twelve weeks after sowing, pods were separated before the remaining haulm was measured and divided into three equal parts for the drying methods, sun dried samples were spread under the sun, air dried were spread under a open ventilate area, while the oven dried were placed inside the oven at 65°C, until moisture reaches 6% by using conventional moisture determination method, after which they were oven-dried at 65°C to constant weight, and were milled for chemical analysis.

#### **Chemical analyses**

##### **Proximate and Fibre analysis**

Proximate analysis was analyzed according to AOAC (2016) and fibre fractions according to the procedure of Van Soest *et al.* (1991). Cellulose was calculated as the difference between ADF and ADL while hemicellulose was calculated as the difference between NDF and ADF.

##### ***In vitro* gas production determination**

*In vitro* gas production: The process of determining *in vitro* gas production was according to the procedure of Anele *et al.* (2011). The 200±0.05 mg of the milled samples were weighed into 100 mL glass syringe fitted with silicon tubes as the only substrate (n = 5). Five syringes of incubation solution without substrate were also included as blanks to assist in estimating the net gas volume. Suction tube was used to collect rumen contents from three White Fulani cattle with an average weight of 140 kg at Cattle Production Venture FUNAAB. The rumen contents were mixed and sieved with 4 layers of cheesecloth under a continuous flushing with carbon dioxide (CO<sub>2</sub>). Macro and micro elements, reduction and resazurin dye solutions were mixed with distilled water. These solutions were mixed in the ratio 2:1 with the rumen fluid. This served as a source of inoculum. Then, 30 mL of the inoculum was drawn into each syringes. Air in the inoculum was eliminated by tapping and pushing the piston in the syringes upward. Controls (blanks) containing 30 mL buffered rumen fluid only were included in triplicates for correction of gas produced from rumen fluid due to the presence of small particles in the fluid. The syringes were placed in a refrigerated incubator with temperature regulated to 39°C. Each syringe's gas production was recorded at 3, 6, 9, 12, 18 and 24 hrs. of incubation. Each incubation time's gas volume was expressed as mL/200 mg of incubated dry matter (DM).

##### **Methane gas volume determination**

The volume of methane (CH<sub>4</sub>) gas produced was estimated 24 hrs post-incubation following the procedure of Fievez *et al.* (2005). This was done with the use of 5 mL syringe to infuse 4.0 mL of 10 M NaOH into three of the incubated contents in the syringes via the silicon tube just above the metal clip for methane production estimation per treatment. A pop sound was heard immediately the NaOH was introduced connoting absorption of CO<sub>2</sub> such that the left-over gas was considered

as CH<sub>4</sub>. The volume of CH<sub>4</sub> gas produced was also expressed in mL/200 mg DM.

#### ***In vitro post incubation parameters***

After 24 hrs of incubation, *in vitro* dry matter digestibility (IVDMD) was assessed. The syringe's contents were empty into crucibles that had already been weighed, then dried in an oven at 105°C until the weight remained constant. The dried residues were weighed and the following equation was used to determine digestibility:

Initial IVDMD (%) =  $\frac{\text{DM input} - \text{DM residue-blank}}{\text{Initial DM input}} \times 100$

- Organic matter digestibility (OMD) was calculated as:  $14.88 + 0.889 \text{ GV} + 0.45 \text{ CP} + 0.651 \text{ Ash}$  (Obadoni and Ochuko, 2002).
- Short-chain fatty acid (SCFA) was calculated as  $0.0239 \text{ GV} - 0.0601$  (Akinbode *et al.*, 2023).
- Metabolizable energy (ME) was calculated as  $2.20 + 0.1357 \text{ GV} + 0.0057 \text{ CP} + 0.0002859 \text{ EE}^2$  (Obadoni and Ochuko, 2002).

Relative methane gas volume (%) =  $\frac{\text{Methane}}{\text{Total gas}} \times 100$

Where, GP is 24 hrs. net gas production (mL 200 mg/DM):

CP = Crude protein content of substrate

Ash = Ash content of substrate

EE = Ether extract

#### ***Statistical analysis***

Data collected was subjected to two-ways analysis of variance and the treatment means was separated using Duncan's Multiple Range test using SAS (2014) package.

## **Results and discussion**

### ***Results***

#### ***Effect of drying methods on the proximate composition (%) and fibre fractions (%) of Bambara groundnut haulm***

Table 1 shows the effect of drying methods on the proximate composition (%) and fibre fractions (%) of Bambara groundnut haulm. There were significantly ( $p < 0.05$ ) different in the observed parameters for proximate composition except ether extract, however the observed parameters for fibre fractions was not significantly ( $p < 0.05$ ) different expect for cellulose. The highest dry matter content (93.60%) was observed in oven dry method with air dry method having the least value of (83.10%) for air dry matter, however the highest content of crude protein (17.40%) and cellulose (19.64%) were observed in air dry method while sun drying method had the highest value (20.87%) for ash content.

#### ***Effect of drying methods on in vitro gas production and Post incubation parameters of Bambara groundnut haulm***

Table 2 shows the effect of drying methods on the *in vitro* gas production and post incubation parameters of Bambara groundnut haulm. There were significantly different ( $p < 0.05$ ) on the parameters except for Metabolized energy, insoluble fractions (b), rate of gas production (b) and Lag time. At the end of the incubation period, oven dried sample had the highest gas production, CH<sub>4</sub>, CO<sub>2</sub>, OMD and SCFA however, air dried sample had the highest value (72.45%) of *in vitro* dry matter digestibility.

## **Discussion**

Higher dry matter was observed in oven dried and sun dried 91.60% and 93.60% respectively, which could be as a result of low moisture content in the sample. The dry matter obtained in air dried (81.10%) was similar to that of 89.86% reported by Adebayo *et al.* (2019). Specifically, the oven-dried samples exhibited the highest dry matter (DM) content at 93.60%. This observation aligns with previous research indicating that oven drying, due to its controlled and consistent

heating environment, is effective in reducing moisture content efficiently (Adewole *et al.*, 2016). High DM content is crucial for enhancing the shelf-life and preventing microbial spoilage of stored legumes (Smith and Jones, 2018). Crude protein (CP) content was higher in air dried sample (16.60%) but has lower content in oven dried and sun dried sample similar to that of Adebayo *et al.*, (2019), the lower crude protein in sun and oven dried could attributed to influence the sun and heat on protein content which might perhaps lead to denaturing, however the range of CP recorded in this studies was well above the threshold of 70 g kg DM required by rumen microbes to build their body protein which was similar to the report of Ojo *et al.*(2024) who report similar CP content for silages made from *Megathyrsus maximum* with *Albizia saman* parts . Ruminant forage intake and rumen microbial activity would be negatively impacted below this threshold. The range of ash content observed in this studies was higher than those reported (Yashim *et al.*, 2012) in the previous findings. Conversely, sun drying resulted in the highest ash content (20.87%), suggesting better retention of mineral constituents under this method. Sun drying, being a natural and gradual process, often preserves heat-sensitive minerals more effectively compared to high-temperature drying methods (Kumar *et al.*, 2017). The elevated ash content in sun-dried samples may indicate a concentration effect due to moisture loss, which could enhance the nutritional mineral profile of the groundnuts (Oladele *et al.*, 2019).

**Table 1: Effect of drying methods on the proximate composition (%) and fibre fraction (%) of Bambara groundnut haulm**

| Parameters     | DM                 | EE    | Ash                | CP                  | NDF   | ADF   | ADL   | HEMI  | CELL               |
|----------------|--------------------|-------|--------------------|---------------------|-------|-------|-------|-------|--------------------|
| Drying methods |                    |       |                    |                     |       |       |       |       |                    |
| Sun Dry        | 91.55 <sup>b</sup> | 2.60  | 20.87 <sup>a</sup> | 16.33 <sup>b</sup>  | 58.40 | 27.61 | 15.67 | 30.79 | 11.94 <sup>b</sup> |
| Oven Dry       | 93.60 <sup>a</sup> | 5.00  | 11.70 <sup>b</sup> | 16.60 <sup>ab</sup> | 56.57 | 27.61 | 15.00 | 28.95 | 12.61 <sup>b</sup> |
| Air Dry        | 83.10 <sup>c</sup> | 5.33  | 11.00 <sup>b</sup> | 17.40 <sup>a</sup>  | 58.92 | 34.53 | 14.89 | 24.38 | 19.64 <sup>a</sup> |
| SEM            | 0.406              | 0.960 | 0.663              | 0.285               | 1.851 | 4.129 | 0.995 | 4.081 | 2.844              |
| P-Value        | 0.00               | 0.170 | 0.000              | 0.047               | 0.660 | 0.443 | 0.841 | 0.280 | 0.045              |

<sup>abc</sup>... means in the same column with different superscripts are significantly different (P <0.05), SEM = Standard error of mean, DM: Dry matter, EE: Ether extract, CP: Crude protein, OM: Organic matter, CHO: Carbohydrate content, NDF: Neutral detergent fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, HEMI: Hemicellulose, CELL: Cellulose

**Table 2: Effect of drying methods on *in vitro* gas production and Post incubation parameters of Bambara groundnut haulm**

| Parameters     | x12hr              | X 24hr             | CH <sub>4</sub>   | CO <sub>2</sub>    | IVDMD               | OMD                | ME    | SCFA              | b      | c     | Lag   |
|----------------|--------------------|--------------------|-------------------|--------------------|---------------------|--------------------|-------|-------------------|--------|-------|-------|
|                | mL/200mgDM         |                    |                   |                    | %                   |                    | mj/kg | (umol/g)          |        |       |       |
| Drying methods |                    |                    |                   |                    |                     |                    |       |                   |        |       |       |
| Sun drying     | 8.01 <sup>b</sup>  | 16.00 <sup>b</sup> | 6.17 <sup>b</sup> | 10.17 <sup>b</sup> | 64.48 <sup>b</sup>  | 50.04 <sup>a</sup> | 15.20 | 0.32 <sup>b</sup> | 98.00  | 0.01  | 2.23  |
| Oven dry       | 13.50 <sup>a</sup> | 24.50 <sup>a</sup> | 9.33 <sup>a</sup> | 15.17 <sup>a</sup> | 69.52 <sup>ab</sup> | 51.75 <sup>a</sup> | 15.80 | 0.53 <sup>a</sup> | 54.00  | 0.03  | 2.19  |
| Air dry        | 7.50 <sup>b</sup>  | 13.83 <sup>b</sup> | 3.67 <sup>c</sup> | 9.50 <sup>b</sup>  | 72.45 <sup>a</sup>  | 42.17 <sup>b</sup> | 15.16 | 0.27 <sup>b</sup> | 14.00  | 0.11  | 1.86  |
| SEM            | 0.47               | 1.15               | 0.37              | 0.78               | 1.53                | 1.18               | 0.56  | 0.03              | 470.05 | 0.03  | 0.16  |
| P-Value        | 0.000              | 0.001              | 0.000             | 0.004              | 0.027               | 0.003              | 0.674 | 0.001             | 0.424  | 0.412 | 0.662 |

<sup>abc</sup>... means in the same column with different superscripts are significantly different (P <0.05). SEM: standard error mean, CO<sub>2</sub>: Carbon dioxide, CH<sub>4</sub>: Methane, IVDMD: *in vitro* dry matter digestibility, OMD: Organic matter digestibility, ME: Metabolized energy, SCFA: short-chain fatty acid, b: insoluble fractions, c: rate of gas production and Lag time.

*In vitro* gas production was highest in oven dried and lowest in air dried sample. The difference in the gas production may be attributed to the different drying methods which might have affected their chemical composition and hence their digestibility. A higher gas production showed that a higher content of digestible nutrients in the legumes which allows rumen microbes to degrade them more quickly. According to Aderinboye *et al.* (2020), higher gas production has been reported to produce short chain fatty acids that will increase the supply of carbohydrates. At the end of the incubation period (x 24hrs), table 2, the values of gas production obtained from this study for the each drying methods was similar to that of Akinyemi *et al.* (2020) for the same drying methods on some browse plants. The post incubation parameters short chain fatty acid (SCFA) metabolizable energy (ME) and organic matter digestibility (OMD) are good indication of forage quality and digestibility because gas production is a reflection of the generation of SCFA and microbial mass (Getechew *et al.*, 1999). Gas volume had direct relationship with metabolizable energy of feed intake (Blummel and Becker, 1997) and growth (Blummel and Ørskov, 1993). The value (15.16 -15.80 MJ/KgDM) for metabolizable energy in this study was higher than that of (7.66-13.00 MJ/KgDM) of Babayemi (2006) for foliages and fruits of *Enterolobium cyclocarpum* during dry season. The value (51.75%), table 2 of OMD of this study was similar to that of Babayemi (2006) for foliages and fruits of *Enterolobium cyclocarpum* (51.42 %) and as well similar to the value (50.48%) to those of Babayemi and Bamikole (2006a) for *Tephrosia candida* and Guinea grass mixture and spent tea leaf (Babayemi *et al.*, 2006). The value of SCFA in  $\mu\text{mol}$  of this study (0.22-0.57) was lower than (0.69 -1.68) of Babayemi (2006) for foliages and fruits of *enterolobium cyclocarpum* but was similar to those of *Tephrosia candida* and guinea

grass mixture of Babayemi and Bamikole (2006) and spent tea leaf of Babayemi, *et al.* (2006). The lower value of SCFA obtained in this study compared to that of Babayemi (2006) for foliages and fruit of *Enterolobium cyclocarpum* may be due to anti-nutritional factors in browse species.

## Conclusion

Oven drying enhances DM, OMD, SCFA, and gas production in Bambara groundnut haulm, making it nutritionally superior but environmentally challenging due to higher CH<sub>4</sub> and CO<sub>2</sub> emissions, however, air drying samples can be adopted for improved crude protein and improve IVDMD but reduces OMD and SCFA.

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