KAMPALA INTERNATIONAL UNIVERSITY
KIU
Journal of Science, Engineering and Technology
KJSET Research Article

Effects of biodegradation of Sesbania Pachycarpa DC leaves on some Physico-Chemical and nutritional characteristics of the digestates

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Paper history:
Received 23rd May 2024
Accepted in revised form 4th July 2024

Keywords
Biomass, Degradation, *Sesbania pachycarpa*, Proximate, Amino acids, Nutritional

Abstract

The effects of biodegradation of Sesbania pachycarpa leaf biomass on some physico-chemical and nutritional characteristics of the digestates was investigated. The fermented and unfermented substrates were replicated trice and assayed using recognized methods. Percentage moisture content and leaf biomass of harvested Sesbania pachycarpa leaf significantly and non-significantly ranged from 61.82 - 69.63% and 0.76 - 0.78g/m^2/day respectively. Temperature and pH increased from 25°C and 5.2 to 34.0°C and 7.5 at 24hrs, gradually decreased and stabilized at 26.5°C and 6.5 after 72 hours of degradation. The crude lipids and nitrogen free extract increased from 3.01 to 5.23% and 40.64 to 42.37% respectively. There were %reductions in crude protein(11.02%), crude fiber(19.38%), ash(1.17%) and moisture(15.38%). All amino acids generally decreased except aspartic acid with 212.14% increase after degradation. Total essential and non-essential amino acids decreased by 4.36% and 54.08% respectively. There were % reduction in total aromatic amino acids (41.94%), Sulphur amino acids (91.77%), acidic amino acids (10.73%), basic amino acids (23.96%), and hydroxyl amino acids (54.01%). Nutritional parameters index, protein efficiency ratio, and amino acid score also reduced, except biological value (13.64%) and metabolizable energy (4.34%). The foregoing revealed the biochemical potentials of the biomass consequent upon the biodegradation process.

Nomenclature and units

MC= Moisture Content (%)
LBP= Leaf Biomass Production (g/m2/day)
M.E= Metabolizable Energy (Kcal)
EAAI= Essential Amino Acid Index
BV= Biological Value (%)
PER= Protein Efficiency Ratio
NI= Nutritional Index

1.0 Introduction

Plant are natural sources of useful biochemical compounds (primary and secondary metabolites) for human and livestock. Most of the present-day plants are unknown and grossly underutilized (Bhat and Karim, 2009). The annual large volume of agro-wastes produced globally, not only portend great environmental and health hazards (Olagunju *et al.* 2013), but also provide veritable potential sources of useful industrial by-products, and feed stocks. Their high fiber component is the main limitation (Jahromi *et al*. 2011). These organic substrates could be converted into biofuels, agrochemicals, animal feeds and human nutrients (Olagunju *et al*. 2013).

The continuous use of chsemical fertilizer has remained a major contributor to change in biogeochemical, eutrophication, greenhouse emissions among others, due to their unsustainable practices which majorly leading to soil and environmental degradation (Amundson *et al*., 2015).

Solid state fermentation could decrease and eliminate anti-nutritional factors (ANF) from plant-based ingredients (Shi *et al*., 2015). The products find beneficial application in aqua-feed production and plant proteins as well, with enhanced nutritional properties. (Khodanazary *et al*., 2013; Shi *et al*., 2015). The effects of the process depend on the fermenting microorganism(s) involved, and the nature of fermentation (Khodanazary *et al*., 2013). The biodegradation process (SSF) involves the mixing of fermenting microbes with appropriate ingredients in a dry state at a very low water activity for fermentation to occur (Pandey, 2003). The technology improves the value chain of otherwise wastes biomass and other organic substrates, in the absence of free water or low moisture condition, to stimulate and sustain growth of fermenting microbes (Pandey *et al*., 2001). It promotes favorable condition for hyphae of especially filamentous fungi to thrive on the substrates and ramify the surfaces.

Sesbania pachycarpa a member of the family Fabaceae: (Papilionoideae) is endemic in West Africa as a weedy species used by farmers in semi-arid zones (Baoua, *et al*., 2011). The different plant parts are used for different purposes ranging from therapeutic (treatment of malaria, helminthiasis febrile body aches, gastritis, wounds, varicose ulcers, ear infections, dracunculiasis and so on), antipicultural (Fafioye, 2005), antioxidant activity (Atawodi, 2005; Ouattara *et al*. 2011), antibacterial in the treatment of syphilis, bacterial infection (Nadembega *et al*., 2011). Ouattara *et al* (2020). reported alkaloids, tannins, saponosides, polyphenols compounds, flavonoids, steroids, coumarins and triterpenes as phytochemical compounds obtained from aqueous leaf extracts of *Sesbania pachycarpa.* These have been reported to have the anti-nutritional properties, apart from its antimicrobial properties, when incorporated as fodder to ruminants.

Reduction of anti-nutritional factors cereal and leguminous crops and plant materials is of great interest. Fermentation technology provides a major approach in this regard (Samtiya *et al.*, 2020). The method has shown some successes in anti-nutrients reduction of protease inhibitors, phytic acids and tannins (Coulibaly *et al*., 2011; Simwaka *et al*., 2017).. Muetzel *et al* (2003), reported barley and saponin-rich *Sesbania pachycarpa* leaf mix affected the growth of *F. succinogenes* and *Ruminococcus flavefaciens*, but inhibited *Ruminococcus albus*'s growth. Also, triterpene-rich saponin decreased the rumen fungi population density, with *Ruminococcus flavefaciens* not affected by the saponins (400 mg/l) (Guo *et al*., 2008).

Li *et al* (2009) showed triterpenoid-rich saponins gave a better antimicrobial activity at low pH in vitro, showing the effect of saponins could be varied by the pH in the rumen based on concentration and nature of the diets. It also selectively affects specific rumen microbes and their metabolic activities. The inhibitory effects of saponnins on enzymes such as amylase, glucosidase, trypsin, chymotrypsin and lipase have been reported (Lee *et al*., 2015). Many plant residues and by products such as rice bran, cassava bagasse, coconut oil cake, soybean cake, among others are being used as substrates for the biotechnological process. (Pandey *et al*., 2001). During the process, hydrolytic exo-enzymes secreted by the synergistic microbes from the cells, support the release of carbon source and other nutrients which promote microbial biosynthesis (Bhavsar *et al.*, 2010.).

Consequent on the foregoing, this study was conducted to evaluate the performance of biodegradation of *Sesbania pachycarpa* Leaves on some physico-chemical and nutritional characteristics of the digestates

2.0 Materials and Methods

2.1. Experimental site and preparation of plant materials

The trial was conducted in two phases at two locations: the field work, involving growing of *Sesbania pachycarpa* at the botanical nursery of the University of Jos, Nigeria (latitudes of 90301 to 100N and longitude 80301E, and it is about 1.25 km above sea level, 6 km above background (Alao and Adeoye, 2004). The *Sesbania* plants were raised on four replicate blocks (5.55 m x 1.50 m). A Randomized Complete Block Design (RCBD) was used to arrange sixteen plots within each block, at a spacing of 50 cm between plant and 1.0 m between blocks. Watering was uniformly done, with adequate supply on a daily basis.

2.2. Determination of moisture content and biomass production

The leaves of *S. pachycarpa* plants were harvested fifteen (15) weeks after planting. The moisture content of the harvested leaves was assessed by taking their fresh weights and thereafter oven-dried to constant weight, using the hot air oven at a temperature of 60ºC. The percentage moisture content was evaluated using the formula in equation (1) below

$$\%\, MC = \frac{A-B}{A} \times \frac{100}{1} \quad \text{.....(1)}$$

Where % MC = percentage moisture content; A = Fresh weight of leaf; B = Dry weight of leaf

The leaf biomass production was determined, using the relationship described by Chomini (1997) in the formula equation (2) below:

$$LBP = \frac{C}{D \times E}\ (g/mday) \text{ .....(2)}$$

Where LBP = Leaf Biomass Production; C = weight of dried leaf (g); D = land area (block or total plots, m^2); E = cultivation time (day).

2.3. Sample collection and fermentation

The harvested leaves of *Sesbania pachycarpa* were aseptically conveyed to the laboratory using sterilized black polythene bags. After air-drying for 3 weeks and pulverized, the samples were fed into a 5000ml beaker, soaked with distilled water (in 1:5 w/v ratio) and boiled for 5 minutes. The boiled content was emptied into a 5L conical flask and buffered with 0.1 M $NaHCO_3$ before seeding with 3.0g sheep faeces (Chomini, 1997). This procedure in triplicates was incubated at 26^0C for 72hrs (Dhembare *et al*., 2015). After 72hrs of degradation, the samples were centrifuged at 5000rpm (Chomini, 1997), filtered with the supernatant disposed, while the residual substrate was oven dried at 80^0C to fixed weight and milled into fine powder for proximate and amino acid assays (Onyimba *et al*., 2010; Chomini *et al*., 2020).

2.4. Determination of proximate and amino acid composition

One hundred grams (100g) of powdered fermented and unfermented *Sesbania* leaves samples (FSL and USL respectively) were subjected to methods of A.O.A.C (2005), for proximate analysis. These included tests for crude protein (CP), crude lipids(CL), total ash (TA), crude fiber(CF), moisture content (MC), nitrogen free extract (NFE), total solids (TS) and volatile solids(VS) as described by Chomini *et al*.(2019).
The metabolizable energy contents (ME) of the FSL and USL samples were determined by the methods of Pauzenga, (1985), adopted by Dairo *et al.* (2017). This involved calculating the M.E. value for each of the samples, using the formula equation (3):-

$$M.E. = 37 \text{ x } \% \text{ CP} + 81.8 \text{ x } \% \text{ CL} + 35.5 \text{ x } \% \text{ NFE} \ldots\ldots\ldots (3)$$

Where: % CP = Percentage crude protein (from proximate analysis) ; % CL = Percentage crude lipid (from proximate analysis); % NFE = Percentage nitrogen free extract (from proximate analysis).
The amino acid composition of FSL and USL samples was analyzed by methods described Spackman *et al*. (1958); Ugoh and Akueshi (2007). This involved defatting, hydrolysis, and analysis of amino acids of the hydrolysates, using a Technicon Sequential Multisample (TSM) Amino Acid Analyzer.

2.5. Determination of nutritional quality

The amino acids profiles formed the bases for determination and comparing the nutritional qualities of both unfermented and fermented samples of *Sesbania parchycarpa*. Essential amino Acid Index (EAAI) was obtained as described by Amza *et al*. (2013), modified in equation(4) below:

$$EAAI = \sqrt[9]{\frac{his \text{ x } lys \text{ x } val \text{ x } met \text{ x } try \text{ x } leu \text{ x } ileu \text{ x } phe \text{ x } thr(TEA)}{his \text{ x } lys \text{ x } val \text{ x } met \text{ x } try \text{ x } leu \text{ x } ileu \text{ x } phe \text{ x } thr(SEAE)}} 100 \ldots\ldots (4)$$

Where TEA = test sample essential amino acids; SEAE = standard essential amino acids for (egg or casein).

Biological value (BV) was determined based on Oser (1959), as adopted by Ijarotimi and Keshinro(2013) in equation(5):

$$BV = 1.09 \times EAAI - 11.7 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where EAAI = Essential amino Acid Index (EAAI).

The protein efficiency ratios (PER) for both unfermented and fermented samples were evaluated by the regression model of Alsmeyer *et al.* (1974), reported by Mune *et al*. (2013), in equation 6 below.

$$PER = -0468 + 0.454 \times (Leu) - 0.105(Tyr) \ldots\ldots\ldots (6)$$

Where Leu = % leucine content of test samples; Tyr = % tyrosine content of test samples

The nutritional index(NI) of both unfermented and fermented samples were determined by formula in the equation (7) described below (Crisan and Sands, 1978; Bagirei et al, 2022),

$$Nutritional\ Index(\%) = \frac{EAAI \text{ x } \% \ Protein}{100} \ldots\ldots\ldots (7)$$

2.6. Data Analysis

The statistical significance was determined by analysis of variance (ANOVA), using SPSS 16, to test the significance. Significant means were separated with LSD test

3.0 Results and Discussions

3.1. The percentage moisture content and leaf biomass production

The percentage moisture content and leaf biomass production of harvested *Sesbania pachycarpa* leaf ranged from 61.82 - 69.63% and 0.76 - 0.78g/m^2/day respectively(Table 1). There was an increase in temperature of the fermentation medium with pH from 25°C and 5.2 to 34.0°C and 7.5, within the first twenty-four hours (24hrs). These gradually declined with the fermentation time up till the end of the trial, with 26.5°C and 6.5, as the terminal conditions at 72 hrs (Figure 1).

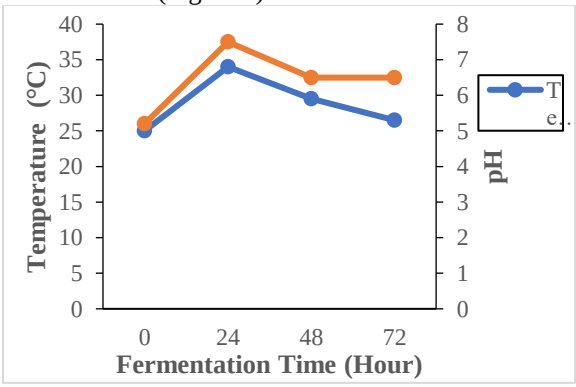

Figure 1: Effects of Solid-State Fermentation on Temperature and pH of the Fermentation Medium

Table 1: Leaf Biomass Production of Sesbania pachycarpa and Moisture Content from Different Dimensions of Ridges

S/N	Ridge Dimension (M)	Weight of Harvested Leaves (g) Fresh	Dry	Moisture (%)	Biomass (g/m2/day)
1	5.55×1.50	2354.80±0.73^{a}	771.80±8.00^{a}	67.25±0.90^{a}	0.77±0.02^{a}
2	5.45×1.49	2311.20±2.16^{b}	763.10±8.41^{a}	66.98±1.45^{b}	0.78±0.07^{a}
3	5.40×1.39	1798.80±2.65^{c}	686.70±3.43^{b}	61.82±0.91^{c}	0.76±0.13^{a}
4	5.65×1.55	2633.30±7.80^{d}	799.80±9.02^{c}	69.63±1.81^{a}	0.76±0.12^{a}
	LSD	12.98	14.04	2.46	0.18

The figures are means ± S.D (where n = 3). Means with unidentical superscripts in the same column significantly different (P<0.05).

3.2. The proximate analysis and amino acid content of fermented Sesbania leaf

The proximate analysis of fermented *Sesbania* leaf (FSL) gave increased values of crude lipids and nitrogen free extract from 3.01 to 5.23% and 40.64 to 42.37% respectively. There were percentage reductions in crude protein, crude fibre, ash and moisture content, with 11.02%, 19.38%, 1.17%, and 15.38% as percentage decreases respectively below those of the unfermented *Sesbania* leaf (USL)(Table 2).

Cysteine (14.55%) and methionine (1.13%), and glutamic acid (4.70%) and cysteine (0.51%) recorded the highest and lowest amino acids values, before and after fermentation respectively. The 72 hours of fermentation of *Sesbania* leaf effected a general reduction in amino acid profile, ranging from 13.29 to 96.49%.

However, only aspartic acid, had an increase from 1.40±0.05% to 4.37±0.33, representing a 212.14%(Table 3)

Table 2: Proximate Composition (%) of Fermented and Un-fermented Sesbania Leaves

	Sample		
Composition (%)	**FSL**	**USL**	**%EF**
Moisture Content	6.99±1.42^{a}	8.26±1.00^{a}	- 15.38
Crude Fibre	7.53±0.92^{a}	9.34±0.82^{ab}	- 19.38
Total Ash	10.13±0.74^{b}	10.26±0.91^{b}	- 1.27
Crude Protein	26.48±1.16^{c}		
Crude lipid	5.23±0.20^{d}	29.76±1.90^{c}	- 11.02
Nitrogen Free Extract (NFE)	42.37±0.94^{e}	3.01±0.02^{d}	73.75
LSD	**1.65**		
		40.64±0.92^{e}	4.26
		1.83	

The figures mean ± S.D (n =3). Means with unidentical superscripts in the same column significantly different (P<0.05). FSL= Fermented *Sesbania* Leaves; USL= Un-fermented *Sesbania* Leaves; %EF= Percentage Effects of Fermentation.

Table 3: Amino Acid Composition of Fermented and Un-fermented Sesbania Leaves Leaves (g/100g Protein)

Amino Acid	FSL	USL	% Effects	FAO/WHO Ref.Standard (1973,1991)
Alanine	2.08±0.16	4.16±0.11	-50.00	6.1
Tyrosine	1.31±0.11	2.25±0.33	-41.78	6.3
Methionine	0.78±0.01	1.13±0.01	-30.97	2.5
Glutamic Acid	4.70±0.33	8.76±0.30	-46.35	14.7
Isoleucine	1.31±0.03	3.29±0.02	-60.18	4.2*
Aspartic Acid	4.37±0.33	1.40±0.05	212.14	7.7
Glycine	1.92±0.02	3.94±0.10	-51.27	2.2
Threonine	1.84±0.14	4.10±0.15	-55.12	3.4
Phenylalanine	2.47±0.13	4.26±0.12	-42.02	6.3
Lysine	3.50±0.41	4.42±0.22	-20.81	5.8
Valine	1.58±0.03	3.54±0.32	-55.37	3.5
Cysteine	0.51±0.01	14.55±0.61	-96.49	2.0*
Leucine	3.12±0.21	6.76±0.82	-53.85	6.6
Proline	1.29±0.01	2.24±0.07	-42.41	10.7
Histidine	1.24±0.01	1.43±0.11	-13.29	1.9
Serine	1.66±0.02	3.51±0.13	-52.71	7.7
Arginine	2.72±0.51	3.96±0.05	-31.31	5.2

* = FAO/WHO 1973; − = reduction; FSL= Fermented Sesbania Leaves; USL= Unfermented Sesbania Leaves.

3.3. Effects of solid-state fermentation on some nutritional parameters

The total amino acids(TAA) for the fermented *sesbania* leaf (FSL) and unfermented *Sesbania* leaf (USL) substrates were 36.40 and 73.70 respectively, representing a reduction of 102.47% due to 72hrs of fermentation. The total essential and non-essential amino acids of FSL (15.84 and 20.56) and USL (28.93 and 44.77) indicated 4.36% and 54.08% reduction due to the fermentation process. In the same vein, there were reductions in the total aromatic amino acids (TArAA), sulphur amino acids(TSAA), acidic amino acids (TAcAA), basic amino acids (TBAA), and hydroxyl amino acids(THyAA) with 41.94, 91.77, 10.73, 23.96 and 54.01% as percentage reduction respectively. However, the ratio of these parameters to the TAA gave % effects of FSL of 91.09% (%TEAA/TAA), 80.71% (%TAcAA/TAA), 53.94% (TBAA/TAA), 17.55% (%TArAA/TAA) over those of USL. While the %effects of %TNEAA/TAA(7.03%), %TSAA/TAA(83.36%) and %THyAA/TAA(6.97%) were in favor of USL.

Other nutritional parameters such as essential amino acid index (EAAI), nutritional index(NI), protein efficiency ratio (PER), and amino acid score (AAScore) gave negative effects due to fermentation, except biological value (BV) and metabolizable energy (ME) with 13.64% and 4.34% respectively(Table 4) TAA= Total Amino Acids; TEAA= Total Essential Amino Acids; TNEAA= Total Non-Essential Amino Acids; TArAA = Total Aromatic Amino Acids; TSAA = Total Sulphur Amino Acids; TAlAA = Total aliphatic Amino Acids; TAcAA = Total Acidic Amino Acids; TBAA = Total Basic Amino Acids; THyAA = Total Hydroxyl Amino Acids; EAAI = essential amino acid index; BV = biological value; NI = nutritional index; PER = protein efficiency ratio; AAScore = Amino acid score; ME = Metabolizable energy.

Table 4: Effects of fermentation on some nutrition parameters

S/n	Nutritional Parameter	USL	FSL	% EFFECTS	RV
1	TAA	73.70±3.15	36.40±1.16	102.47	-
2	TEAA	28.93±1.67	15.84±0.90	-4.36	33.9
3	%TEAA/TAA	39.25	43.51	91.09	-
4	TNEAA	44.77±3.92	20.56±1.33	-54.08	-
5	%TNEAA/TAA	60.75	56.48	**-7.03**	-
6	TarAA	6.51±1.01	3.78±0.58	-41.94	6.3
7	% TArAA/TAA	8.83	10.38	17.55	-
8	TSAA	15.68±6.71	1.29±0.14	-91.77	2.5
9	%TSAA/TAA	21.27	3.54	-83.36	-
10	TAcAA	10.16±2.18	9.07±0.09	-10.73	-
11	%TAcAA/TAA	13.79	24.92	80.71	-
12	TBAA	9.81±1.31	7.46±0.94	-23.96	-
13	%TBAA/TAA	13.31	20.49	53.94	-
14	THyAA	7.61±0.30	3.50±0.09	-54.01	-
15	%THyAA/TAA	10.33	9.61	**-6.97**	-
16	EAAI	1.446	0.1871	-87.07	-
17	BV	-10.12	-11.50	13.64	-
18	NI	0.43	0.05	-88.37	-
19	PER	2.36	0.81	-65.68	-
20	AAScores	79.99	42.35	-47.06	-
21	ME	2790.72	2911.71	4.34	-

3.4 Discussion

The pattern of leaf biomass production of *S. parchycarpa* showed no significant effects ($p>0.05$) across ridges, indicating that the sowing time was adequate to generate the recorded biomass. This supported the findings of Mafongoya and Jiri (2015), who posited that biomass production was not affected by both dry and wet season in relation to soil nitrogen. Chanda *et al* (2020), reported that biomass yield of *Sesbania* could be enhanced by different adoptable cultural practices. These include different sowing dates (Chanda *et al*., 2018a), plant population densities (Srivastava & Kumar,2013; Chanda & Sarwar, 2017b) as determined by different spacing, genetic makeup (Chanda & Sarwar, 2017a; Naidu *et al*. 2017), longer sunshine hours, adequate soil moisture (Chanda *et al*. 2018b), as well as application of nitrogen fertilizer (Chanda *et al*., 2020).

The sudden rise in pH within the first 24hours from 5.2 to 7.5, was similar to the report of Chomini *et al* (2020), on solid state fermentation on post-harvest cowpea leaves. They observed pH stability of 6.65 at 96hrs of fermentation (HOF), which was slightly higher than the stabilized pH value of 6.5 between 48 and 72 HOF, obtained from the present study. Awasthin (2015), explained that the drastic change in pH values was due to organic acids production such as citric and lactic during the anaerobic process, leading to pH reduction. Conversely the increase in pH was indicative of degradation of protein and the organic acids, forming resulting into amino acids and peptide formation. Zehra *et al* (2020), posited that the pH value of the fermentation medium could affect enzyme production by controlling the solubility, cell membrane permeability and nutrients ionization.

The initial increase in temperature from 25°C to 34°C within the first 24 hrs, which gradually decreased to 29.5°C and 26.5°C at the 72hrs. corroborated the observations of Zehra *et al* (2020), who observed a sudden temperature rise from 25°C to 34°C in the digestive medium of *Aspergillus fumigatus* MS16 inoculated banana Peels for the production of xylanase and pectinase. They further observed a drastic reduction in pectinase levels at higher temperature of 40°C**.** Naves *et al* (2012), monitored the effects of temperature range of 20 to 70 °C on phytase activities, and found an optimal phytase production at pH 5.5 and 50 °C. Coradi *et al* (2013), reported stability of lipase enzyme activity within temperature and pH range of 20 to 40 °C (60 min, pH 6.0) 5.0 to 8.0 (60 min, 28 °C), maintaining that the enzymes are generally stable within the neutral pH and alkaline range (Lima *et al.*, 2004).

The reduction in crude protein (CP) content of the fermented materials corroborated reports of Bartkiene *et al* (2020), showing 29.4, 26.8 and 21.2% reduction after 24, 48 and 72 hours of solid-state fermentation of industrial by-products of barley respectively. They related their findings to the capability of the lactic acid bacteria to secret proteinases, lysing proteins in the substrates (Gardini *et al*., 2016). The pattern of reduction across the fermentation periods also reflected the enzymes' activity as well as possible inhibitions (Bartkiene *et al.*, 2020). On the contrary, Onweluzo and Nwabugwu (2009) and Chomini *et al* (2020), reported varying %increases in CP, due to SSP of different organic substrates. The observed increase in % fat was attributed to biosynthesis of long chain fatty acids from acetyl co-enzymes A and other complex unsaturated lipids during fermentation (Oso *et al.*, 2017). They indicated as high as 50% increase in %fat due to urea buffered fermentation of cassava root tubers materials. Oboh and Elusiyan (2007), suggested that the rise in fat content subcould be due to microbial oil secretion during fermentation.

According to Olugosi *et al* (2019), decrease in %ash in the range of 16.9 - 25.9% due to solid state fermentation, was attributable to depletion of mineral elements during the process. The present study recorded 1.27%. Other researchers have also in their opinions, posited that the residual ash content is a function of moisture loss and organic matter removal (2016). Olusola-Makinde, and Oluwafemi (2020), attributed the decrease to mineral utilization by microbes during metabolism. Conversely, Aro and Aletor (2012), reported an increase in %ash of fermented cassava wastes, which they attributed to hydrolysis of chelating phytate-rich wastes.

The decrease in crude fiber (CF) content (19.38%) of fermented *Sesbania* leaf corroborate the observations by Alemawor *et al* (2009), Adeyeye *et al* (2017) and Olugosi *et al* (2019), who reported 17.08%, 14.83% and 14.7% as %decreases in CF of fermented cocoa pod husk respectively. This reflected the ability of the fermenting microbes to secret appropriate cellulose and hemicellulose-lysing enzymes during the metabolic process (Alemawor *et al*., 2009). This invariably improved the digestibility of the fermented substrates by monogastrics **(**Olugosi *et al*., 2019).

Olusola-Makinde and Oluwafemi (2020), also reported reduction in %fiber content of fermented Bush Mango seed (*Irvingia gabonensis*).

Ubwa *et al* (2014), reported enhancement of nitrogen free extract (NFE) resulting from urea treatment preceding solid state fermentation of rice milling waste. Aro and Aletor (2012), indicated a 2.10% and 48.97% as percentage increases in NFE of solidstate fermentation on cassava starch residues and naturally fermented cassava peel. This increase in NFE contents were thought to result from microbial enzymatic actions on the crude

fiber fraction of the organic waste into their soluble carbohydrate units. Chomini *et al* (2020), corroborated this observation with an increase of 5.0% in NFE content due to 96hours of fermentation. Onyimba *et al* (2010), however pointed out that any decrease in the NFE content was attributable to microbial assimilation especially at the point of cessation of fiber metabolism.

Apart from aspartic acid, all other amino acids recorded decreases in contents after 72 hours of fermentation. These observations were similar to those of Bartkiene *et al* (2020), during a 72 hour of solid-state fermentation of industrial barley waste. They also affirmed a reduction in total amino acid (TAA), which was attributed to the assimilation during microbial metabolism as well as enzymatic conversion, to different amino acid as described by (Khan *et al*., 2018). Osman (2011), reported a general reduction in amino acids across fermentation time, except for methionine and tyrosine, after 24 hours of fermentation. These could be attributed to inherent toxicants and anti-nutritional factors in leguminous substrates. These secondary metabolites such as tannins, phytic acid, protease and trypsin inhibitors, saponins, metal chelates, cyanogens, isoflavonoids, phytoalexins, flatus factors, etc., antimicrobials against the naturally fermentative microbes (Pariza & Johnson, 2001; Chaves-López *et al*., 2020). They may also interfere with protein and carbohydrates metabolisms and mineral bioavailability (MacDonald *et al*., 2012).

According to Adebayo *et al*. (2019), the increase in glutamic and aspartic acids might be engendered acid hydrolysis of glutamine and asparagine, resulting to glutamic and aspartic acids production, alongside ammonium (NH4+) ions secretion. The contrast could have informed the present observations. The decrease in total essential amino acid (TEAA) of FSL below the USL agreed with the report of Muhammed and Oloyede, (2006), showing excess production of non-essential amino acids over the essential, with a depleting effect on the latter and total amino acid (TAA) content of FSL (Ugoh & Akueshi, 2007). Most of the essential amino acids did not compare favourably with FAO/WHO standards (FAO/WHO, 1973, 1991), they were all limiting, with relatively lower values than the referenced standards.

These observations could have been occasioned by inherent antimicrobials in the substrates. Ouattara *et al* (2020), reported several phytochemicals in the leaf of *S. pachycarpa*. Several studies have shown the antimicrobial effects of these secondary metabolites against many fermentative microbes (Patra & Saxena, 2009). Saponin has been reported to show adverse antimicrobial effects on many microbial strains including rumen and bacteria and fungi, methanogenic as well as (Patra & Saxena, 2009). Cieslak *et al* (2013), revealed that saponins mitigate methanogenesis mainly by reducing the population of the as well as by being directly toxic to methanogens and other fermentative microbes. The mitigating effects of tannins were reported (Bodas *et al*. 2012; Cieslak *et al*.2012), to cause 2 to 58% reduction in fermentation product and by products. According to Tavendale *et al*. (2005), inhibition of growth of methanogens resulted from the bacteriostatic and bactericidal effects of condensed tannins (CT). This may be due to deactivation (Pellikaan *et al*., 2011), suppression and reduction of methanogens (Bhatta *et al*., 2009; Goel and Makkar, 2012). Jouany and Morgavi (2007), observed that terpenoids or phenols interference of ion transport (electrons) through the cell membrane, mitigate protein translocation, phosphorylation and other enzymatic processes. Consequently, it effects stratification of microbial population, due to internal structural alteration, resulting in decrease in total microbial population (Kongmun *et al*. 2011).

The essential amino acid indices (EAAIs) were lower than 13.62% reported values for fermented popcorn flour (Ijarotimi & Keshinro, 2011). All the nutritional parameters were relatively lower than most the standard reference values. Some workers (Oser, 1959; Esan, *et al*. 2018), reported EAAIs value of 90% considered suitable as food when the values are around 80% and otherwise when less than 70%. According to FAO/WHO/UNU (2007), the BV provides a basis to forecast residual nitrogen as an index of utilizable fraction of a protein. The P-PER is one of the quality parameters used for protein evaluation. A good quality protein must have value of 2.5 or more (AOAC, 2005; Ijarotimi & Keshinro, 2011). These observations are closely related to the initial explanation on the effects of substrate phytochemicals on the digestive microbes.

5.0 Conclusions

The leaf biomass production of *Sesbania pachycarpa* was not affected significantly by plot dimension. The degradation process effected significant variations in pH and temperature of fermentation medium. There were percentage reductions in crude protein, crude fiber, ash and moisture content, while crude lipids and nitrogen free extract significantly increased due to 72 HOD. All amino acids generally decreased except aspartic acid with 212.14% increase after degradation. Most of the essential amino acids were limiting, with consequential reductions in most of the nutritional parameters except biological value (BV) and metabolizable energy. These consequently engender a necessity for pretreatment of the leaf substrates to reduce the inherent antimicrobial bioactive compounds, which could have impeded the process.

Acknowledgements

The supports and assistance of the entire laboratory and the Herbarium staff are highly commendable

Declaration of conflict of interest

The authors declared no conflict of interest.

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