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Effects of Solid State Fermentation on some Physicochemical and Nutritional Properties of Post-Harvest Cowpea (*Virgna unguiculata* (L)Walp) Leaves

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Abstract

The effects of solid state fermentation on some physical characteristics, proximate and amino acid profile of post-harvest cowpea (*Virgna unguiculata* (L) Walp) leaves were investigated. Pulverized samples of the post-harvest materials were incubated at 26^{0}C for 96 hours, followed by urea and trichloroacetic acid treatments. Triplicate samples of fermented and unfermented materials were subjected to standard procedures to determine variations in weight, pH, proximate and amino acids profile. There was a reduction in weight, which was significant ($p<0.05$) with increase in fermentation time. pH stabilized at 6.62 and 6.65 at 96 hours of fermentation (HOF), before and after urea treatment, respectively. Fermented samples showed significant increase ($p<0.05$) in crude protein (37.30%), crude fat (95.69%), total ash (75.73%) and nitrogen- free extract (NFE) (5.00%) over the unfermented ones. However there were percentage reductions in crude fibre (46.60%) and moisture content (61.95%) after fermentation. The Total Amino Acids (TAA) increased from 49.64 ± 0.87 to 98.90 ± 1.70 with a general increase in all amino acids except proline and cysteine having 12.72% and 10.06% as percentage reductions, respectively. Some essential amino acids (methionine, phenylalanine and tyrosine) and non-essential amino acids (serine and proline) were limiting. The findings unveiled the feed supplement potentials of the fermented materials for use in livestock and pharmaceutical industries in Nigeria.

Keywords: Fermentation, Nutritional Value, Post-Harvest, Cowpea
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Introduction

One of the major bottle-necks in livestock production in developing countries is the availability of rich crop residues and forages. Livestock farming in the subtropics are raised on poor forages during the summer and prolonged drought, when aridity is encroached, resulting in huge economic loss (Nitis, 1999). The use of forage and browse legumes as alternative protein nutrient sources had been examined (Baloyi et al., 2008; Contreras-Govea et al., 2009; Andrade et al., 2017). Endogenous protein supplies few amino acids under normal feed conditions (O'Conner et al., 1993), as most insoluble proteins often escape along with faecal matter (Tedeschi et al., 2001). The solid-state fermentation (SSF) of agriculture residues and feedstock has become attractive due to its potential to enhance nutritional value and increase nutrient bioavailability (Wang et al. 2018). Fermentation, a veritable biochemical option (Jones, 1993), has been reported to effect a number of useful changes on bio-substrates. These range from improved nutritive values (Wakshama and Akueshi, 2009), enhanced flavor (Wakshama and Akueshi, 2008), impacted colour, taste and aroma (Ishiwu et al., 2015), improved protein, amino acid and lipids (Wakshama and Akueshi, 2009), influenced crude fibre content (Ogbonna and Popoola, 1997; Olatunde and Ekerigin, 2007; Verduzco-Oliva, and Gutierrez-Uribe, 2020), as well as enhancement of bio-colours and pigmentation during solid state fermentation (Mhalaskar et al., 2017). According to Ray and Swain (2011), two types of anaerobic digestion are identified, depending on whether hydration is involved or not. In view of the numerous aforementioned potentials of the biomass degradation processes, this study therefore reports on the effects of biodegradation on some physical characteristics, proximate and amino acid profile of post-harvest cowpea leaves.

Materials and Methods

Experimental Site

The trial was conducted in the microbiology laboratory of the University of Jos, Nigeria. Jos is located at latitudes of $9^{0}30^{1}$ to 10^{0}N and longitude $8^{0}30^{1}$E, and it is about 1.25 km above sea level, 6 km above background. The climate is distinctively of the tropics, with an average yearly rainfall of 1,250mm, which culminate between July and August. It has an average yearly temperature of 22^{0}C, with a mean value of 19.4^{0}C and 24.5^{0}C for winter and summer, respectively (Alao and Adeoye, 2004).

Sample Collection and Fermentation

Post-harvest Cowpea (*Vigna unguiculata* L. Walp) leaves were obtained from a local farm in Jos. They were aseptically brought into the laboratory in black polythene bags. Into a 5000 ml beaker were loaded pulverized samples of the post-harvest leaves, which were previously sun-dried for 2 weeks. These were soaked with distilled water (300g/1500ml w/v) and allowed to boil for five minutes. The content was transferred then into a 5 L conical flask and buffered with 0.1 M $NaHCO_3$ and thereafter inoculated with 3.0 g sheep faeces. The set up in triplicate was incubated at 26^{0}C for 96hrs (Onweluzo and Nwabugwu, 2009, Dhembare et al., 2015). After 96 hrs of fermentation, the samples were flooded with 225 ml of 0.1 M urea solution with pH assessment at 2 hrs intervals. The procedure was repeated until the pH stabilized slightly below neutral point (pH=6.98) (Chomini, 1997; Kolapo et al., 2007; Mrudula, and Murugamma, 2011). At this point, the samples were treated with 0.1 M trichloroacetic acid (TCA) at a concentration of 1.0g/20.0 mL (W/V) and subsequently centrifuged at 5000 rpm. The filtered supernatant was disposed of, followed by repeated treatment of the residual substance 5 more times, to remove non-proteinous nitrogenous compounds. The treated samples were oven- dried at 80 ^{0}C to constant weight and pulverized into fine powder for proximate and amino acid analysis (Ugoh and Akueshi, 2007; Wakshama and Akueshi, 2009).

Proximate and Amino Acid Profile Determinations

One hundred grams (100g) of the pulverized samples of fermented and unfermented post-harvest cowpea leaves (FPCL and UPCL respectively) were subjected to standard procedures as described by A.O.A.C (2005). For proximate composition analysis, the methods as described by Wakshama and Akueshi (2009) were adopted. The amino acid profile was assayed based on the methods of Spackman et al. (1958); Ugoh and Akueshi (2007).

Data Analysis

Statistical significance was determined with SPSS 16, using analysis of variance (ANOVA). A follow up procedure was performed with LSD to assess the outstanding means.

Results

There was a steady decrease in weight with an increase in fermentation time. An initial increase in pH from 6.68 ± 0.03 to 7.95 ± 0.02 was recorded after 24 hours of fermentation (HOF), which decreased to 6.62 ± 0.02 at 96 HOF before urea treatment and 6.65 ± 0.05 after urea treatment (Figures 1 and 2).

After 96 HOF, proximate analysis revealed a significant (P<0.05) increase in crude protein, crude fat, total ash and nitrogen- free extract (NFE) contents of the fermented post-harvest cowpea leaf (FPCL), with 37.30%, 95.69%, 75.73% and 5.00% as percentage increases, respectively, over those of unfermented post-harvest cowpea leaves (UPCL). However, the latter had higher crude fibre and moisture contents (15.15 ± 0.12% and 12.72 ± 0.11%) than those of the former (8.09 ± 0.11% and 4.84 ± 0.14%), representing 46.60% and 61.95% reductions, respectively, due to fermentation (Table 1). The nutritive values of the fermented leaves increased significantly (P<0.05) as reflected in all the amino acid contents, with Histidine recording the highest percentage increase of 190.79%, while Proline and Cysteine had 12.72% and 10.06% as percentage reductions, respectively (Table 2). Also there was an increase in the percentage essential amino acids from 48.41% to 50.52%, while a decrease in non-essential amino acid from 51.59% to 49.4% was observed, sequel to 96 HOF.

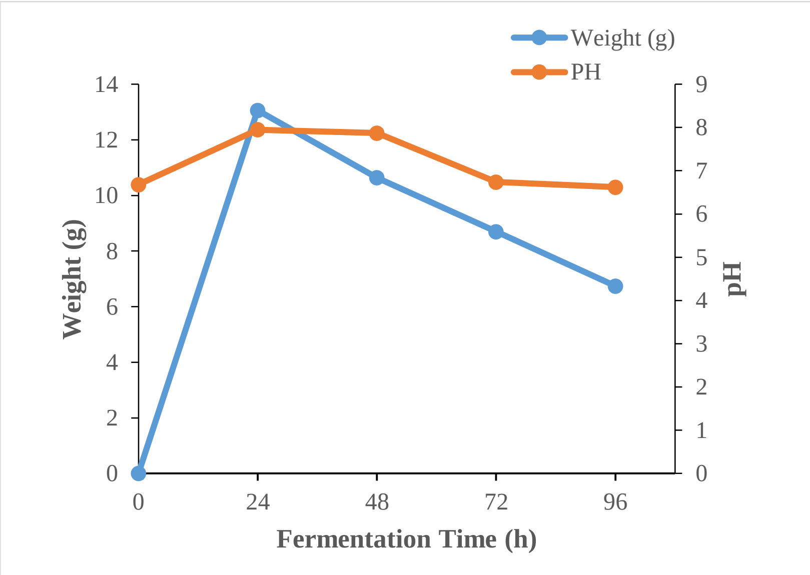

Figure 1: Effect of solid state fermentation on average weight (g) and pH of Cowpea leaves

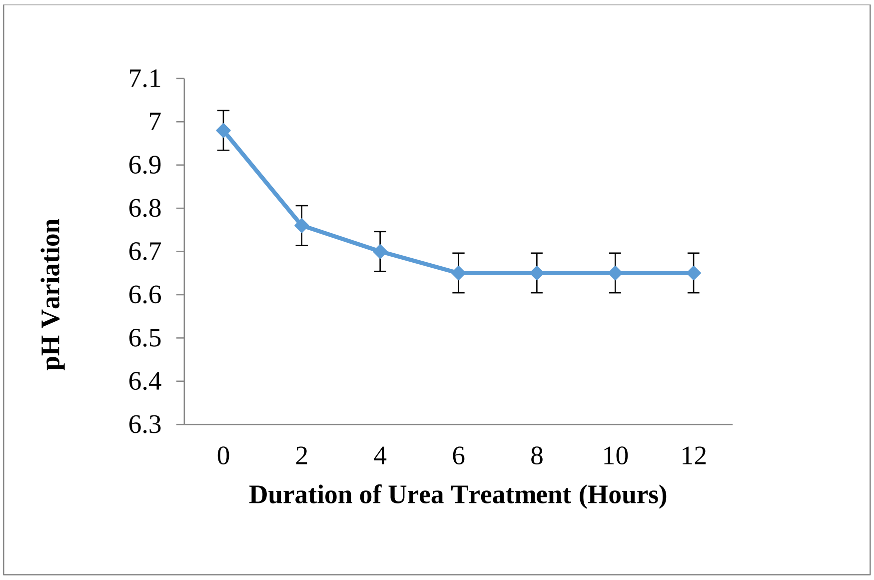

Figure 2: Effect of Duration of Urea Treatment (Hour) on pH Variation

Table 1: Proximate Composition (%) of Fermented and Unfermented Post Harvest Cowpea Leaves

	Sample		
Composition (%)	**FPCL**	**UPCL**	**%EF**
Moisture Content	4.84±0.14[a]	12.72±0.11[a]	- 61.95
Crude Protein	25.03±0.10[b]	18.23±0.12[b]	37.30
Crude Fibre	8.09±0.11[c]	15.15±0.12[c]	- 46.60
Crude Fat	4.09±0.10[d]	2.09±0.11[d]	95.69
Total Ash	8.98±0.14[e]	5.11±0.24[e]	75.73
Nitrogen Free Extract (NFE)	49.10±0.21[f]	46.76±0.16[f]	5.00
LSD	**0.25**	**0.25**	

Values are means of triplicate determination ± S.D; means with different superscripts in the same column differ significantly (P<0.05); FPCL= Fermented Post Harvest Cowpea Leaves; UPCL= Unfermented Post Harvest Cowpea Leaves; %EF= Percentage Effects of Fermentation.

Table 2: Amino Acid Profile of Fermented and Unfermented Post-harvest Cowpea Leaves (g/100g Protein)

Amino Acid	**FPCL**	**UPCL**	**% Effects of Fermentation**	**FAO/WHO Ref. Standard(1973,1991)**
Lysine	6.57±0.10	2.98±0.03	120.47	5.8
Histidine	2.21±0.40	0.76±0.07	190.79	1.9
Arginine	5.69±0.13	2.39±0.05	138.08	5.2
Aspartic Acid	11.14±0.06	4.83±0.13	130.64	7.7
Threonine	5.69±0.05	3.11±0.06	82.96	3.4
Serine	5.32±0.04	2.85±0.03	86.67	7.7
Glutamic Acid	15.08±0.12	5.77±0.14	161.35	14.7
Proline	2.95±0.05	3.38±0.17	-12.72	10.7
Glycine	5.15±0.03	2.67±0.05	92.88	2.2

Alanine	6.35±0.18	2.83±0.03	124.38	6.1
Cysteine	2.95±0.03	3.28±0.02	-10.06	2.0*
Valine	4.77±0.04	1.97±0.02	142.13	3.5
Methionine	1.80±0.03	1.24±0.01	45.16	2.5
Isoleucine	5.52±0.18	2.71±0.01	103.69	4.2*
Leucine	9.79±0.10	4.92±0.02	98.98	6.6
Tyrosine	3.58±0.12	1.60±0.02	123.75	6.3
Phenylalanine	4.34±0.04	2.35±0.01	84.68	6.3
TAA	98.90±1.70	49.64±0.87	99.23	-
TEAA	49.96±1.19	24.03±0.30	107.91	-
%TEAA	50.52	48.41	4.36	-
TNEAA	48.94±0.51	25.61±0.57	91.09	-
%TNEAA	49.48	51.59	-4.09	-

Values are means of triplicate determinants ± SD; * = FAO/WHO 1973; − = reduction
FPCL= Fermented Post Harvest Cowpea Leaves; UPCL= Unfermented Post Harvest Cowpea Leaves; TAA= Total Amino Acids; TEAA= Total Essential Amino Acids; TNEAA= Total Non Essential Amino Acids

Discussion

The stability of the pH at 6.65 was similar to the values reported by Chanjula et al., (2003); Promkot and Wanapat (2003); Suchitra and Wanapat (2008), which were considered optimal for microbial digestion necessitating protein formulation. This is because above the neutral condition, the enzyme activities are not favoured and may retard the metabolic activities, thereby hampering the process (Akinyele et al., 2014). Dhembare et al., (2015), observed an optimal performance of pectinase activity (340.56 µg /ml/sec), at pH 4 at 96 hours of incubation, but did not produce significant increase beyond 5. This was attributed to the preference of *Aspergillus niger* for relatively lower pH for its growth and metabolism. According to Nema et al., (2019), maximum lipase activity of 25.12 U/gds was observed at pH 6, while further increment gave a significant reduction in lipase activity, at pH 7.5 (7.14 U/gds).

The increase in protein content of the fermented material agreed with the reports of Bhalla and Joshi (1994), Adeyemo et al., (1999), Oboh and Elusiyan (2007) and Onweluzo and Nwabugwu (2009), who assessed the effects of fermentation on various plant materials. They explained this increase to be due to extracellular enzyme secretion, leading to microbial biomass (single cell protein) production. Khan et al., (2017), reported an enhancement of protein content of formulated diets with urea- treated groundnut shells. Similarly, Somda et al., (2018), reported higher % crude protein (CP) value with urea supplementation than non-supplemented substrates. Ubwa et al., (2014), indicated 13.94% increase in crude protein of urea treated rice milling wastes over the untreated wastes. This was lower than 37.30% currently reported. This increase in % CP was attributed to an increase in microbial growth/biomass due to favorable fermentation medium (Olugosi et al., 2019). Oboh and Elusiyan (2007), have attributed the observed increase in fat content of the fermented samples to the possibility of microbial oil secretion in course of fermentation. Igbabul et al., (2014), reported an increase in fat from 1.83 to 2.61% of fermented cocoyam flour. Similarly, Oso et al., (2018), revealed 40% and 50% increases in percentage fat due to urea- supplemented fermented peeled and unpeeled cassava root tubers, respectively. They ascribed this increase to microbial metabolism of aliphatic long chain fatty acids from acetyl co-enzymes A and other unsaturated fatty by-products.

The decrease in crude fibre content of FPCL corroborate the findings of Adeyeye et al., (2017) and Ozung et al., (2017), who reported a 14.83% and 57.42% decrease after fermentation of cocoa pod husk, respectively. This reduction was explained as an indication of the ability of microbes to secrete enzymes for degradation of polymeric lignocelluloses. Ubwa et al, (2014), recorded % reduction in crude fibre fraction of urea-treated rice milling waste below the untreated waste values. Oso et al., (2018), attributed the reduction in the urea-treated fermented cassava root tubers to ligno-cellulolytic enzymes secreted by *Aspergillus niger* inoculated to the fermentation medium. The increase in % ash corroborated the findings of Aro and Aletor (2012), who adduced the observed increase to the hydrolysis of chelating phytate-rich cassava waste. Imelda et al., (2008), reported up to 63% increase in crude ash of some fermented agricultural wastes. The observations were opined to be due to the dry matter loss during fermentation, leading to a relatively high unaltered component of the fermented Product. Chomini et al., (2019), reported a % increase ranging from 48.03-763.60%, due to 56 days of anaerobic co-fermentation of poultry droppings and maize cobs. On the contrary, Olugosi et al., (2019), reported a reduction in % ash ranging from 16.9-25.9% with fermentation time, which was attributed to depletion of mineral elements during the fermentation period. Ubwa et al., (2014), observed an increase in nitrogen free extract (NFE) due to fermentation of urea-treated rice milling waste. This, according to Onyimba et al., (2010), indicates higher levels of soluble or near soluble carbohydrates such as sugars resulting from the degradation of cellulose. They posited that the drop in the NFE values was due to the assimilation of the breakdown products by microorganisms especially at a time when there was no further degradation of fibre.

Apart from Proline and Cysteine, FPCL was significantly ($P<0.05$) favoured over UPCL, which according to Muhammed and Oloyede, (2009), Oyarekua and Adeyeye, (2009), accounted for the increase in total amino acids (TAA) of the former over the latter. This corroborated the findings of Muhammed and Oloyede, (2009) first week of fermentation of seeds of *Terminalia catapa* and 72hrs of co-fermentation of sorghum/cowpea by Oyarekua and Adeyeye (2009). Higher values of Glutamic acid, Aspartic acid and Leucine of FPCL were consistent with reports by Wakshama and Akueshi (2009), and Muhammed and Oloyede, (2009). The higher value of Glutamic acid was opined to be connected to and responsible for aromatic and flavour enhancement (as observed), due to protein hydrolysis (Oyarekue and Adeyeye, 2009; Wakshama and Akueshi, 2009). This was described as monosodium glutamate formation, which serves as a major component in food seasoning and condiment (Ishiwu et al., 2015). Adebayo et al., (2019), indicated that increase in glutamic and aspartic acids could have stemmed from acid hydrolysis of glutamine and asparagine, leading to their conversion to glutamic and aspartic acids with a release of ammonium ($NH4+$) ions. However, the increase in total essential amino acid (TEAA) of FPCL over the UPCL contradicted the report by Muhammed and Oloyede, (2006), indicating that essential amino acids had been synthesized at the expense of the non-essential ones, thereby improving the quality of amino acid and total amino acid (TAA) contents of FPCL (Ugoh and Akueshi, 2007). Igwe et al., (2012), described the increase in essential amino acids as an enzymatically mediated process by fermenting microbes, due to chemical constituents breakdown, leading to enhancement of available amino acids. These corroborated with the findings of Bao et al., (2013), indicating an increased total essential amino acids of fermented rice with *Pleurotus eryngii*. Dairo et al., (2017), reported a significant additive effects of the *Pleurotus ostreatus* fermentation of rice brand on lysine and methionine, which supported the current findings. The improved essential amino acids compared favourably with FAO/WHO standards (FAO/WHO, 1973, 1991), with serine, proline, methionine , tyrosine and phenylalanine limiting, with relatively lower values than the referenced standards. This was similar to the observed scenario by Iwuagwu and Ugwuanyi (2014), suggesting the suitability of the fermented product as feed to ruminants, pigs, fish and poultry.

Conclusion

The urea-treated (supplemented) fermentation process has important application. It revealed progressive reduction in biomass with fermentation time. It also led to an increase in crude protein, crude fat, ash and nitrogen-free extract, with 46.6% and 61.95% as % reductions in crude fibre and moisture content, respectively. There were outstanding increases in the contents of all amino acids except proline and cysteine with 12.72% and 10.06% as % decreases, respectively. Consequent upon these findings, it is recommended that further studies be carried out on toxicological and acceptability potential, while other agricultural residues are considered.

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