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Nutritional Quality of Fungal Composted Substrates

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Abstract
The study investigated the nutritional quality of fungal composted substrates. The substrates namely: chicken feather, hair waste, egg membrane, cow horn and hoof were subjected to fungal (fusarium spp) composting for 7 weeks. The substrates were collected and characterized physicochemically and microbiologically. During the composting process, parameters such as temperature, total organic carbon, potassium, phosphorus, rate of degradation, pH and microbial counts, moisture, ash, carbohydrate, crude protein, fibre, lipid and fungal counts; were monitored using standard methods. The results showed that physico-parameters measured showed significant differences ($p<0.05$) among substrates. Total organic carbon (%) before and after composting for substrates 1, 2, 3, 4 and 5 was 4.06 and 2.10, 12.79 and 0.48, 18.02 and 1.29, 20.34 and 62.7 and 1.12, 9.01 and 3.50 respectively. Total protein (%) before and after composting for substrates 1, 2, 3, 4 and 5 was 62.7 and 58.7, 64.6 and 62, 1.60 and 1.50, 26.4 and 23.9, 34.8 and 34.1 respectively. Total heterotrophic fungal counts (10^3CFU/g) before and after composting was; 1.4 and 3.0, 3.0 and 4.0, 2.0 and 5.0, 2.80 and 8.0 and 4.0 and 3.0, respectively. The highest rate of degradation (ROD) was recorded in substrate 2 (chicken feather, 96.17) followed by substrate 4 (94.20). The study suggest that substrates 1 and 2 appear to have higher nutritional values, therefore may be composted for fish and livestock feed formulation.

Keyword: *increasing population, keratin waste, composting*

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I. Introduction

As a result of population increase and changes in living standards, the size of various livestock enterprises has grown to meet food demand. Hence, the number of various wastes generated around the world has increased dramatically (Kumar *et al*., 2017). Many municipal and industrial wastes, primarily from the food industry, include high concentrations of carbohydrates, water, proteins, lipids, and inorganic substances (Karimi *et al*., 2018). Aside from the scarcity of virgin resources, environmental concerns about organic-rich waste management and disposal have long been a source of worry (Singh and Kumari, 2019). To reduce their environmental impact, these organic-rich wastes must be efficiently processed in order to recover their nutrients and minerals and transform them into valuable products. Associated problems resulting from indiscriminate dumping of organic waste into the environment might be solve or reduced by the use of organic waste as a substitute for low-cost, high-quality fish feed (Akankali and Nwafili, 2015).

Additionally, the economy and environment of the country have been impacted by the in-efficient utilization of by-products of industrial and organic-rich waste produced in massive proportions on a daily basis, containing significant levels of nutrients that are recoverable (Chatterjee and Mazumder, 2016). As by-product, enormous quantities of chicken feathers, hair debris, egg membrane, cow horn, and hoof are produced. Incineration or land filling is both problematic methods of disposal. For instance, pollution and disease transmission caused by microbial contamination. They are, nevertheless, made up of materials and components (protein and keratin fibres) that can be transformed into a large and diverse variety of valuable products and materials. In order to mitigate (or alleviate) the negative impact of indiscriminate disposal of these organic materials into the environment, there is need to divert them from the waste stream and employed as resources for feeding, biofertilizer production, bioenergy generation, among others (Ifeoluwa, 2019).

There has been huge success with the utilization of Fungi and actinomycetes to decompose keratin-rich materials like feathers. Fungi are important for their invasion into the body by the keratinases secreted (Li, 2019). On the skin's surface of humans or animals, pathogenic fungi are present; these fungi have distinct characteristics, such as hyphae, that help in keratin degradation (Korniłłowicz-Kowalska and Bohacz, 2011; Tridico *et al*., 2014). Pathogenic fungi that produce keratinases must be avoided in applications because of the need for safety. On the other hand, some non-pathogenic fungus has the ability to breakdown these wastes and hence has the potential to be acceptable for use as a biofertilizer or in animal feed (Bhange *et al*., 2016). Others can be composted; composting involves mixing organic materials to provide a moist, aerobic environment conducive to organic matter degradation and humification (Sinha *et al*., 2010). Furthermore, little is known

about the composition of the microbial population at various phases of composting, which might be crucial in the degradation process.

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As a consequence, it appears that investigating alternate methods is worthwhile. Several microorganisms, including fungi (*Doratomycesmicrosporus, Alternariaradicina, Aspergillus* sp., *Rhizomucor* sp., etc.), actinomycetes (*Streptomyces pactum, S. thermoviolaceus, Thermoactinomyce scandidus*, etc were reported to produce keratinase which is the specific class of proteolytic enzymes cleaving keratin containing substrates (Abdel-Fattah *et al*., 2018). Hence, this study was carried out to understand the biodegrading process of fungi *spp* in composting.

II. Materials and Methods

Experimental design: The non-conventional protein sources used were cow hoof and horn, poultry feather, hair waste and egg membrane with *fusarium* spp as fungi inoculums using a randomized complete block designed (RCBD).

Table 1: Experimental set-up for fungal composting of non-conventional protein sources

Reactors	Substrates	Total solid (g)	Moisture content (g)	Dry solid(g)	Ash content(g)	Volatile solid(g)	Fungal load $X10^3$cfu/g)
1	CHV	10	0.04	9.96	0.03	9.93	1.4
2	FeM	10	0.06	9.94	0.12	9.82	3.0
3	EM	10	0.05	9.95	0.27	9.68	2.0
4	HW	10	0.12	9.88	0.01	9.87	2.8
5	CH	10	0.07	9.93	0.13	9.80	4.0

CHV- Cow hooves, FeM- Feather meal, EM- Egg membrane, HM- Hair waste and CH- Cow horn

Selection and Collection of Non-Conventional Protein Sources

The various substrates were sourced in the University of Port Harcourt environment while Fungi was cultured and collected from a private microbiology laboratory in Port Harcourt, Rivers State, Nigeria.

Physico- chemical and microbiological analysis of compost

Physico- chemical parameters was determined as described by APHA (1998), while cultural methods described by Selvankumar *et al*., (2018) was used to determine fungal load.

Statistical analysis

One-Way analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) version 21 was used to determine various data obtained. The differences between group mean (±SE) was determined using Duncan multiply range test (DMRT) at 5% level of probability using the same software.

III. Results

Biological and physico-chemical parameter monitored during fungal (*Fusarium spp*) composting of substrates

The results of the mean variation of biological and physico-chemical parameter of the substrates are shown in Table 1. Temperature of substrate 3 (29.70±0.04) was significantly higher than substrate 1 (29.54±0.04) but was not different from substrate 2 (29.57±0.04). The percentage rate of degradation (ROD) of substrates 2(68.39±6.22), 3(63.64±6.22) and 4 (62.88±6.22) were significantly different from 1(19.81±6.22) and 5(34.17±0.04) but substrate 1 had the lowest degrading ability. Furthermore, the total nitrogen present in substrates 1(6.31±0.43) and 2 (5.52±0.43), was significantly higher compare to substrates 4 (3.05±0.43), 5 (3.97±0.43) and 2(0.92±0.43) respectively. In addition, substrate 1 for Potassium content ($7.04±0.12^{a}$) was significantly higher. There was significant difference between each substrate for fungal counts.

Table 1: Mean biological and physico-chemical parameter monitored during bio-composting of substrates

Parameters	**Substrates**				
	1	**2**	**3**	**4**	**5**
Temp(^{O}C)	$29.54±0.04^{b}$	$29.57±0.04^{ab}$	$29.70±0.04^{a}$	$29.58±0.04^{ab}$	$29.54±0.04^{b}$
pH	$7.17±0.07^{c}$	$7.72±0.07^{b}$	$8.31±0.08^{a}$	$7.66±0.07^{b}$	$8.37±0.07^{a}$
TOC (%)	$3.27±0.99^{c}$	$4.06±0.99^{bc}$	$6.55±0.99^{ab}$	$7.56±0.99^{a}$	$5.85±0.99^{abc}$

ROD (%)	19.81±6.22^{b}	68.39±6.22^{a}	63.64±6.22^{a}	62.88±6.22^{a}	34.17±6.22^{b}
T-N (%)	6.31±0.43^{a}	5.52±0.43^{a}	0.92±0.43^{c}	3.05±0.43^{b}	3.97±0.43^{b}
K (%)	7.04±0.12^{a}	1.36±0.12^{b}	0.46±0.12^{c}	0.30±0.12^{d}	1.35±0.12^{b}
P (%)	7.49±0.16^{a}	8.15±0.16^{a}	2.11±0.16^{c}	0.16±0.16^{d}	6.29±0.16^{b}
THFC(x10^{3}cfu/g)	3.65±0.25^{bc}	5.64±0.25^{a}	4.20±0.25^{b}	5.96±0.25^{a}	3.26±0.25^{c}

Mean values (mean ± standard error) in same row with different superscript differ significantly different ($p<0.05$). Temp= Temperature, pH= hydrogen ion, TOC= Total dissolve solid, ROD= Rate of degradation, T-N= Total nitrogen, K= Potassium, P= Phosphorus, THFC= Total fungi count.

Biological and physico-chemical parameters during bio-composting of substrates

During the weekly microbial decomposition of substrates, temperature values for cow hoof, chicken feather, egg membrane, hair waste and cow horn ranged from 29.3 to 29.9 ºC, 29.3 to 30 ºC, 29.4 to 30.2 ºC, 29.3 to 29.8 ºC, and 29.3 to 29.9 ºC (figure 1). Values for pH for substrates 1 to 5 ranged from 6.8 to 7.4, 7.3 to 7.9, 7.0 to 7.7, 7.1 to 8.0 and 6.8 to 8.5 respectively (figure 2). Total organic carbon ranged from 2.10 to 4.06, 0.48 to 12.79, 1.29 to 18.02, 1.12 to 20.34 and 3.50 to 9.01 for substrates 1 to 5 respectively (figure 3). Rate of degradation ranged from 0 to 48.3, 0 to 96%, 0 to 93%, 0 to 94% and 0 to 61.2% for substrates 1 to 5 respectively (figure 4). Total nitrogen of cow hoof, chicken feather, egg membrane, hair waste and cow horn ranged from 4.12 to 9.72 %, 1.64 to 10.24%, 0.08 to 0.35% 1.54 to 5.41% and 1.40 to 6.48% respectively (figure 5). Similarly, potassium concentration ranged from 6.8 to 7.50%, 1.3 to 1.40%, 0.09 to 0.81%, 0.28 to 0.31% and 1.3 to 1.39% for substrates 1 to 5 respectively (figure 6). Phosphorous content ranged from 6.98 to 7.98%, 8.04 to 8.56%, 0.10 to 0.14%, 0.10 to 0.22% and 6.10 to 6.90% for substrates 1 to 5 respectively (figure 7). Total fungal counts ranged from 1.4 to 6.0 x10^{3}cfu/g, 3.0 to 9.0 x10^{3}cfu/g, 2.0 to 6.0 x10^{3}cfu/g, 2.8 to 8.4 x10^{3}cfu/g and 2.0 to 4.0 x10^{3}cfu/g for substrates 1 to 5 respectively (figure 8).

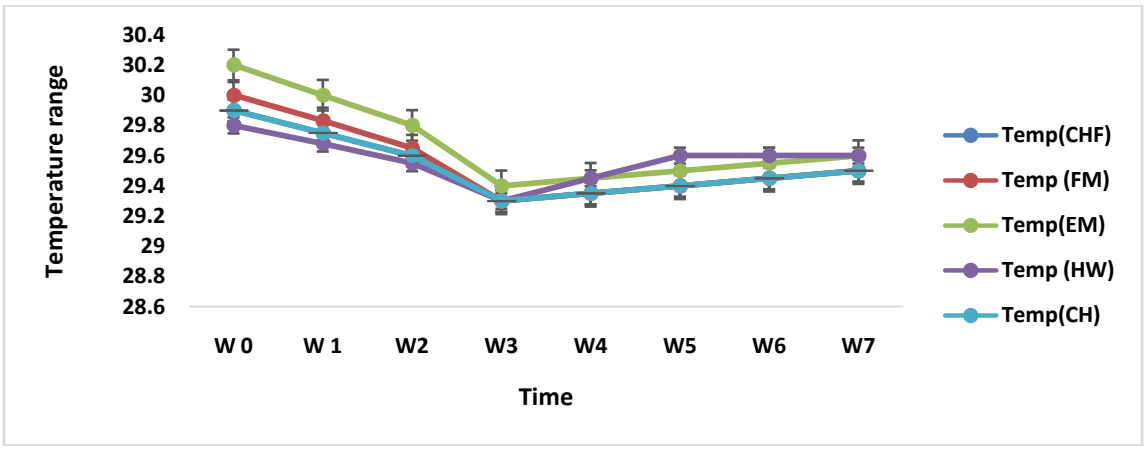

Figure 1: temperature variations during fungal composting of substrates

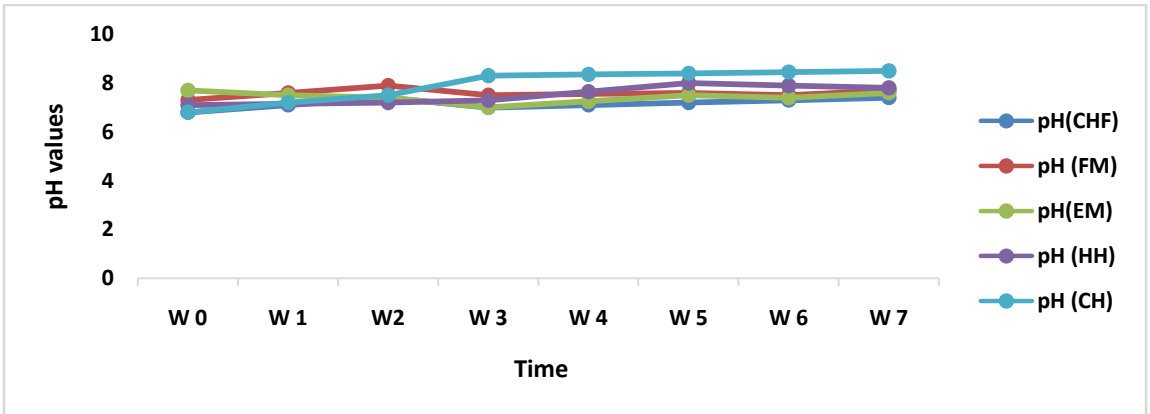

Figure 2: Changes in pH values using fungi spp

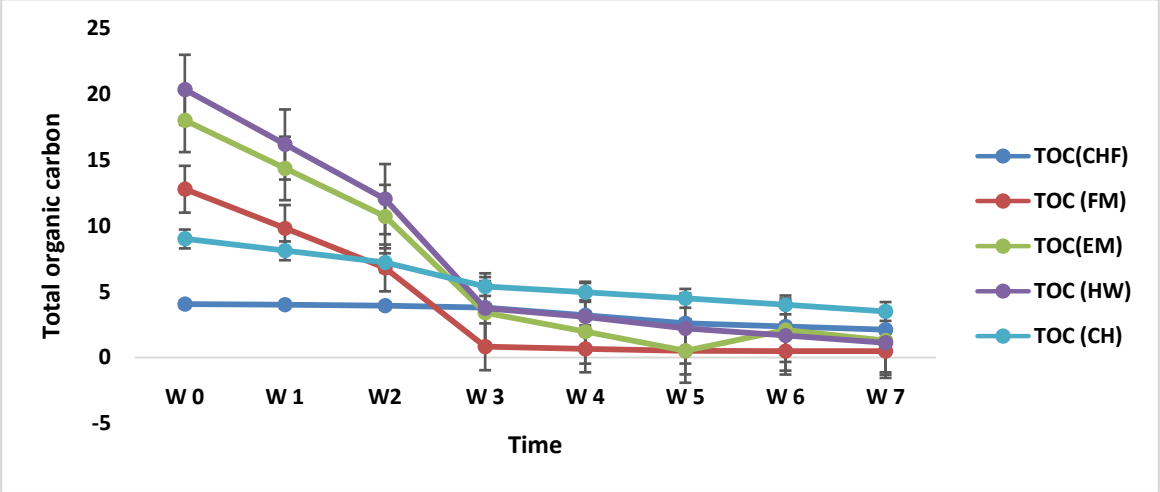

Figure 3: Total Organic Carbon values during fungi spp composting of substrates

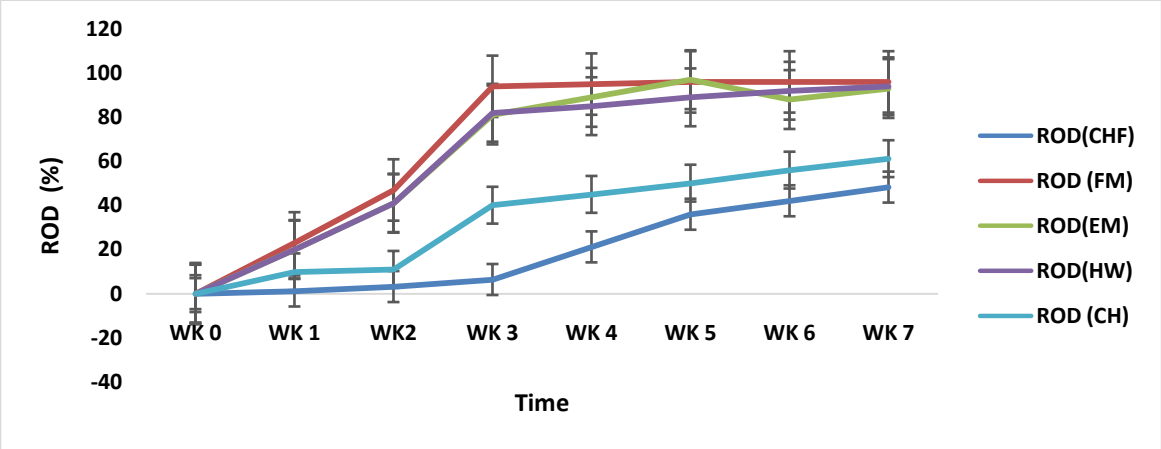

Figure 4: Weekly Rate of Degradation values during fungal spp composting.

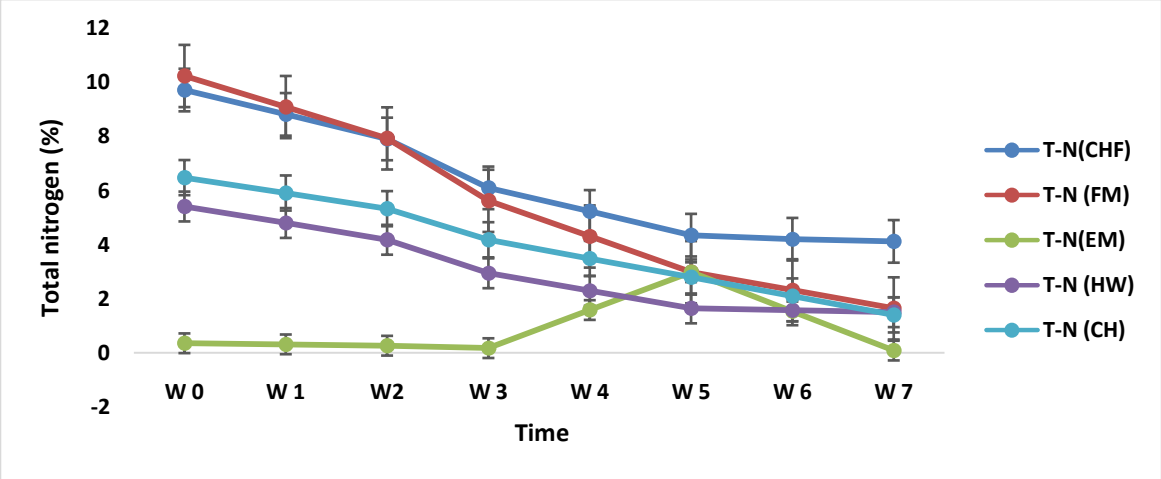

Figure 5: Total Nitrogen values during fungal composting of substrates

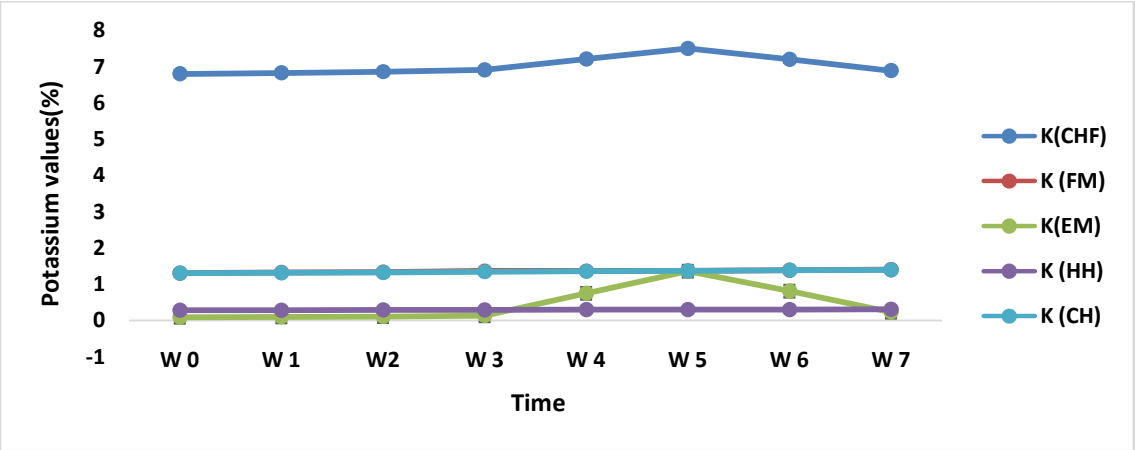

Figure 6: Potassium (K) values during fungal composting of substrates

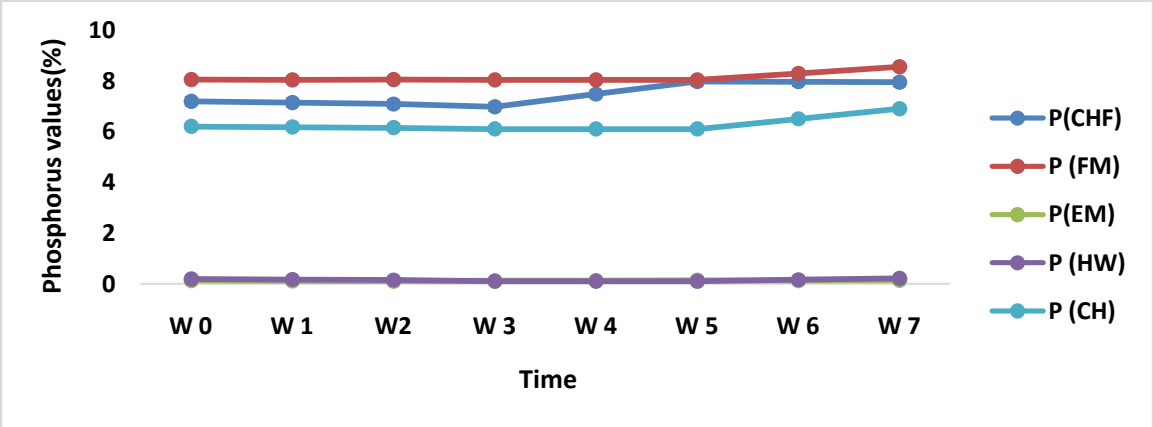

Figure 7: Changes in Phosphorus values during Fungal compostingof substrates

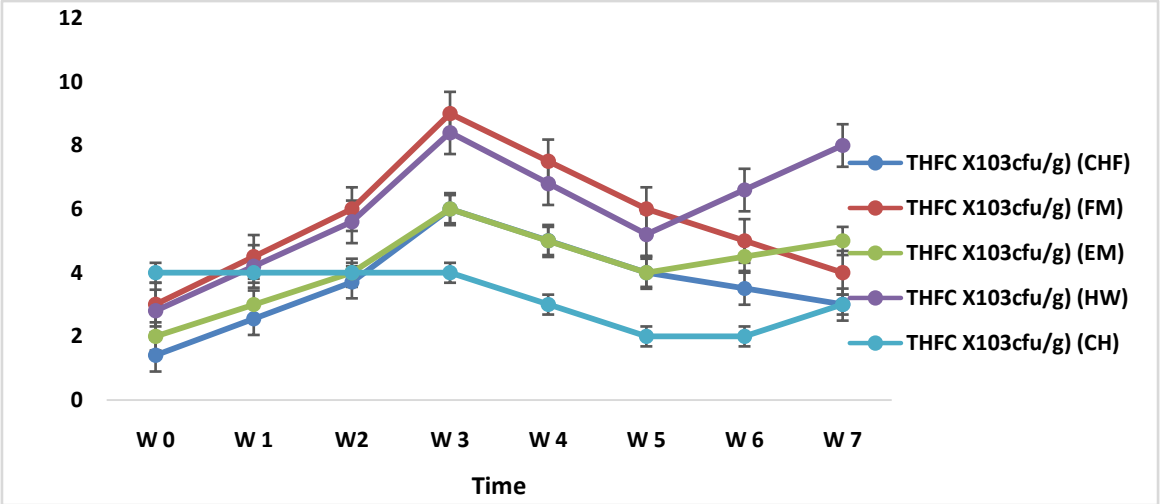

Figure 8: Fungal counts during microbial composting of substrates

Proximate composition of monitored during fungi composting of substrates

The results observed from the proximate composition monitored using fungi spp showed significant differences ($p<0.05$). Moisture content of substrates 2(67.69 ± 5.68^{a}), 3 (61.08 ± 5.68^{ab}) and 4(64.07 ± 5.68^{a}) were not significantly different ($p>0.05$) from substrate 1 (46.57 ± 5.68^{bc}). Ash content of substrates 3 (30.01 ± 0.94^{a}) and 5 (29.94 ± 0.94^{a}) were significant differences from substrates 2 (16.27 ± 0.94^{b}), 1 (8.12 ± 0.94^{c}) and 4(3.97 ± 0.94^{d}). The ether extract content in substrate 1(6.51 ± 0.05^{a}) was significantly higher than substrates 2 (5.80 ± 0.05^{b}), 3 (1.30 ± 0.05^{c}), 4(0.85 ± 0.05^{d}) and 5(0.96 ± 0.05^{d}). Crude protein in substrate 2(63.21 ± 0.19^{a}) was significantly higher compared to all other substrates 1(60.26 ± 0.19^{b}), 3(1.56 ± 0.19^{e}), 4 (24.74 ± 0.19^{d}) and 5

(33.86±0.19^{c}). The crude fibre content in substrate 4 (58.22±0.10^{a}) was significantly different from substrates 1(7.26±0.10^{b}), 3(3.50±0.10^{c}), 2(1.09±0.10^{d}) and 5(0.78±0.10^{e}). Carbohydrate content in substrate 3(58.22±0.73^{a}) was higher compared to substrates 5 (30.76±0.73^{b}), 1 (14.34±0.73^{c}), 2 (7.86±0.73^{d}) and 4(0.36±0.73^{e}).

Table 2: Mean proximate composition of substrate monitored during bio-composting.

Parameters%	Substrates				
	CHF	**CF**	**EM**	**HW**	**CH**
Moisture	46.57±5.68^{bc}	67.69±5.68^{a}	61.08±5.68^{ab}	64.07±5.68^{a}	35.23±5.68^{c}
Ash	8.12±0.94^{c}	16.27±0.94^{b}	30.01±0.94^{a}	3.97±0.94^{d}	29.94±0.94^{a}
Ether	6.51±0.05^{a}	5.80±0.05^{b}	1.30±0.05^{c}	0.85±0.05^{d}	0.96±0.05^{d}
Crude protein	60.26±0.19^{b}	63.21±0.19^{a}	1.56±0.19^{e}	24.74±0.19^{d}	33.86±0.19^{c}
Crude Fibre	7.26±0.10^{b}	1.09±0.10^{d}	3.50±0.10^{c}	58.22±0.10^{a}	0.78±0.10^{e}
Carbohydrate	14.34±0.73^{c}	7.86±0.73^{d}	58.78±0.73^{a}	0.36±0.73^{e}	30.79±0.73^{b}

Mean values (mean ± standard error) withdifferent superscript in the same row are significantly different (p<0.05). Cow hoof (CHF) Chicken feather (CF), egg membrane (EM), hair waste (HW) and cow horn (CH)

Proximate parameter monitored during bio-composting of substrates

Moisture content ranged from 3.9, 4.9, 5.9, 11.8 and 6.9% to 66.5, 93, 80.5, 85.8 and 48% for substrates 1 to 5 respectively (figure 9). Ash content during the composting process ranged from 2.5 to 11.4 , 11.5 to 18.8, 26.5 to 32.5, 0.89 to 5.1 and 12.5 to 36.5 for substrates 1 to 5 respectively (figure 10). The ether extract content ranged from 6.2 to 6.8%, 5.2 to 6.5%, 1.28 to 1.3%, 0.67 to 1.18% and 0.85 to 1.2% for substrates 1 to 5 respectively (figure 11). Crude protein content ranged from 58.7 to 62.7%, 62 to 64.6%, 1.50 to 1.60%, 23.9 to 26.4 and 34.1 to 34.8% for substrates 1 to 5 respectively (figure 12). Crude fibre content ranged from 6.80 to 8.42, 0.94 to 1.28, 3.19 to 4.26, 57.4 to 59.1 and 0.74 to 0.86 for substrates 1 to 5 respectively (figure 13). The carbohydrate content ranged from 13.25 to 15.98, 7.04 to 10.22, 56.61 to 61.44 , 0.30 to 0.63 and 21.91 to 43.74 for substrates 1 to 5 respectively (figure 14).

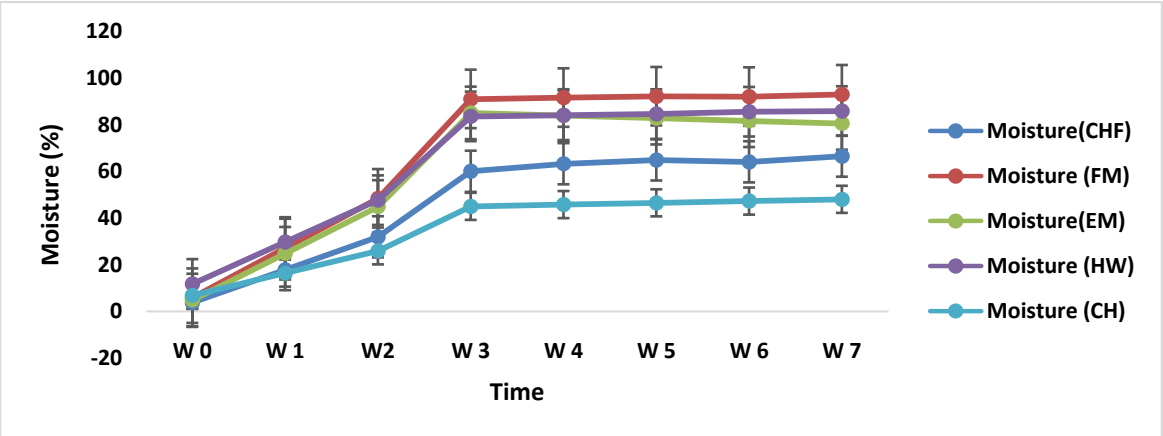

Figure 9: Moisture content monitored during fungal composting of substrates

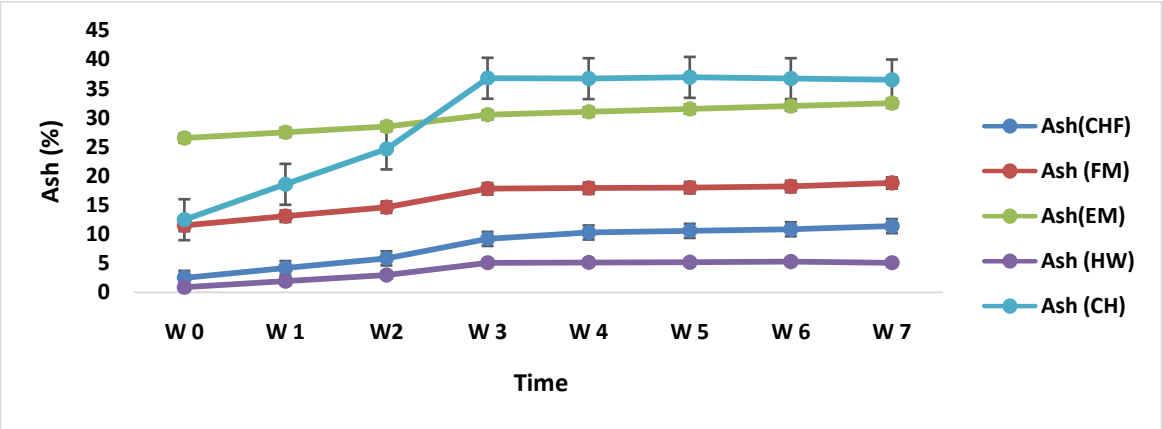

Figure10: Ash content monitored during fungal composting of substrates

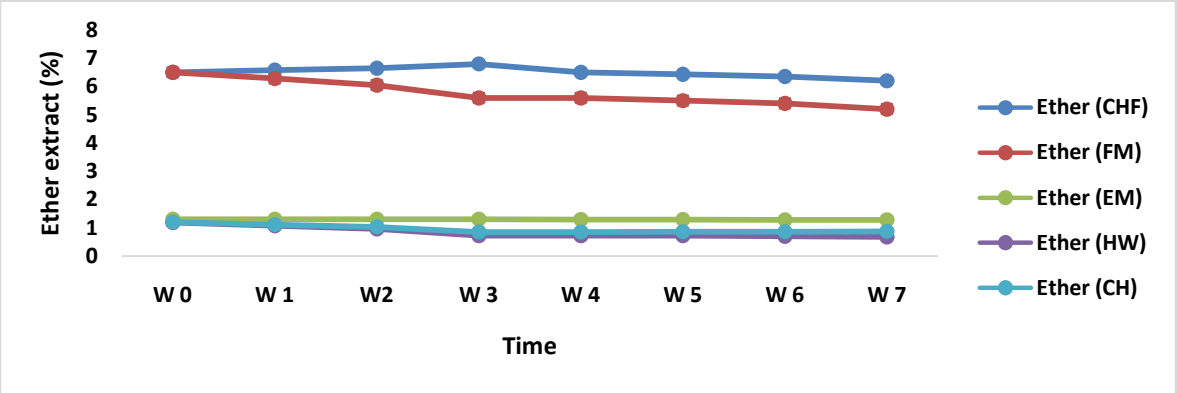

Figure 11: Ether extract content monitored during fungal composting of substrates

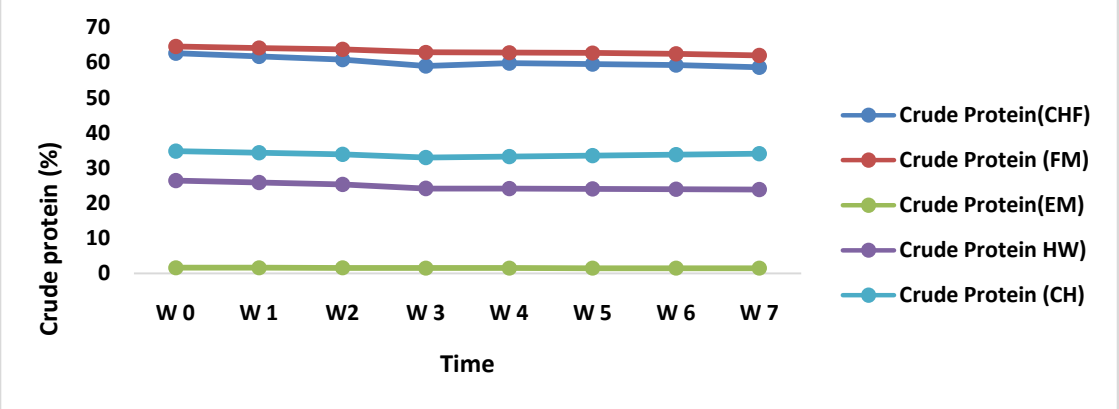

Figure 12: Crude protein content monitored during fungal composting of substrates

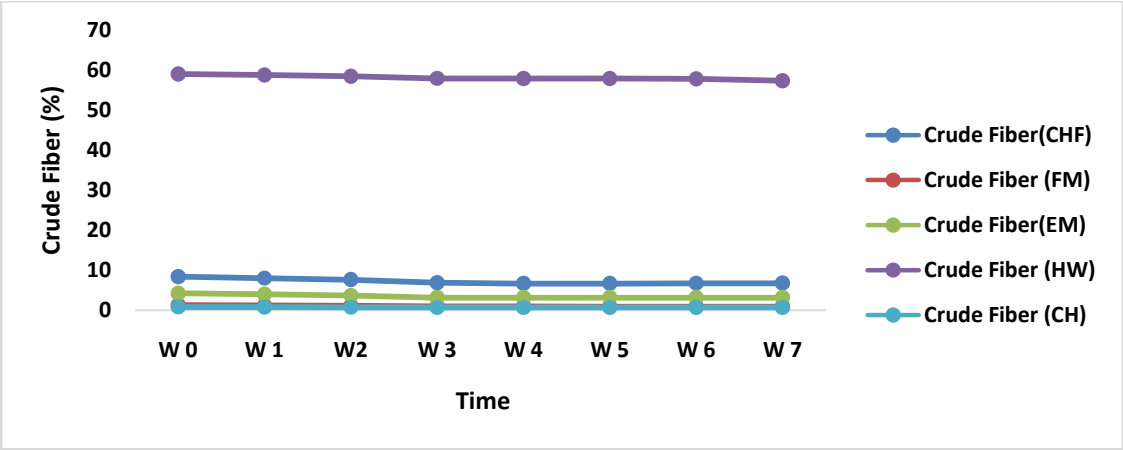

Figure 13: Crude fiber content monitored during fungal composting of substrates

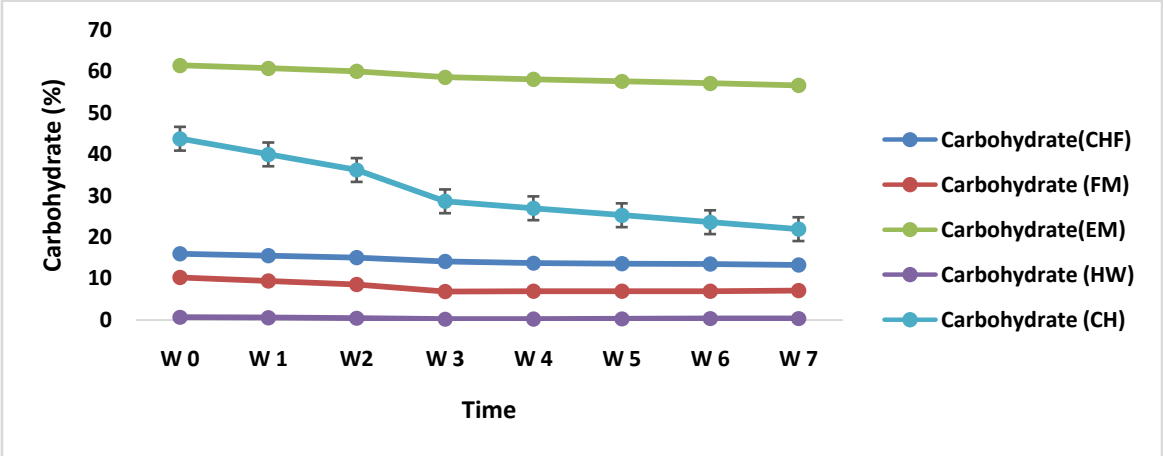

Figure 14: Carbohydrate content monitored during fungal composting of substrates

IV. Discussion

Biological and physico-chemical parameter monitored during fungal composting of substrates

During the composting process, the temperature range of the substrates increased. This may be due to the depletion of organic materials, a lack of air inside the piles, and the degree of enzyme synthesis by the microorganisms that are causing the decomposition. Canet and Pomares (1995) noted a comparable difference in temperature with a maximum temperature of 69.5^{o}C. According to Intagun and Kanoksilapatham (2017) keratinases from fungi are active at low temperatures, with a range of 28-90^{o}C or even higher,). This can be the outcome of an aerobic process or an increase in heat. However, some were lower than others, such as substrates 1 and 5, indicating slow degradation in the compose heap, which may be due to the substrate's particle size.

In order to speed up decomposition, pH values often rise during composting. The rise may be caused by the digestion of keratinaceous waste, according to Ignatova *et al*. (1999), who found this to be the case. For all substrates, the pH value increased from weeks 4 to 7 of the composting process. This might be because fungal development causes the compost pile to become more acidic as a result of the early stage of composting creating more organic acids. Additionally, the production of CO^2 and the loss of nitrogen from organic acids can also be attributed (Lugtenberg *et al*., 2013).

During microbial composting, the total organic carbon in all substrates was significantly reduced. Despite the fact that substrate 5 had low values in comparison to other substrates. However, the amount of total organic carbon decreased during the degradation process. It varies from 0.48 to 20.34%. The findings of (Sekar *et al*., 2015) who reported a range of 11.56 to 41.34% in all the samples are different from those of this study. Composting has a considerable impact on organic carbon loss. The percentage rate of degradation also decreased in accordance with this. The substrates might be broken down, as evidenced by the microbes. Depending on their physical characteristics, certain substrates degrade more quickly than others.

For cow hooves, the rate of degradation varied from 0 to 48%, for feather, from 0 to 96%, for egg membrane, from 0 to 93%, for hair waste, from 0 to 94%, and for cow horn, from 0 to 61.2%. In substrate 3, the overall nitrogen content was lowest. Sekar *et al*. (2015) reported similar findings with ranged from 0.38 to 1.84%. The slow decomposition of organic substrate, which comprises amino sugars and proteins, may be the

cause of the nitrogen content's decline during composting.

The potassium content ranged from 0.09 to 7.50 per week. In comparison to other substrates, substrate 1 had greater potassium content. This finding did not match the variation that (Sekar *et al*., 2015) found, which ranged from 0.65 to 1.97%. Even yet, they had comparable potassium increases during the composting process. The greatest total phosphorus concentrations were found in substrates 2 and 1. The concentration ranged from 0.11 to 8.56 per week. According to Janakiram and Sridevi (2011), total phosphorous concentration steadily increased throughout the composting process and decreased with humification of water solubility of phosphorus. The total number of fungus during the composting process ranged from 1.4 to 9.0 X 10^3 cfu/g t. There was an increase in weeks 6 and 7 which indicated the lag phase of microbial degradation. Microbial count is very essential in that the population of microbes will determine the rate of decomposition.

The amount of moisture in the compost mixture is a significant environmental component that influences the environment in which nutrients necessary for the physiological and metabolic processes of microorganisms may occur. Throughout the composting process, it was seen that the moisture content of the compost was rising. It might be because water was added to the substrates to speed up the microbial degrading process and help in substrate decomposition. Maximum microbial activity was observed to occur in the moisture range of 60–70% by Liang *et al*., (2003). Additionally, moisture has a greater impact on microbial activity in controlled environments than temperature.

All substrates had an ongoing rise in ash content during the composting process. Substance 1 had the greatest ether extract concentration compared to the other substrates. The range of ether content during composting was from 0.67 to 6.8, and the ether content of the substrate decreased in step with that. Although substrate 3 contained the least amount of protein, substrate 2 had the largest amount of crude protein. There was a reduction in crude protein during the composting process; the range of crude protein degradation was from 1.50 to 64.6%. This may be the result of the microorganisms breaking the link during the degradation process and the release of carbon dioxide during aerobic bio-composting (Gupta *et al*., 2019). During the composting process, crude fiber decreased. In comparison to substrates 5, 1, 2, and 4, substrate 3 had larger carbohydrate content. Substrates varied in carbohydrate content from 0.18 to 61.44%. Throughout the composting process, the carbohydrate content decreased. The carbohydrate content of feather meal increased in week 7 despite utilizing fungus to break it down.

V. Conclusion

According to the study, composting with fungus produced the highest levels of protein quality and degradability in the seventh week. With the exception of egg shell membrane, considerable crude protein levels were found after biochemical examinations of the different substrates. The development of environmentally friendly technology for the treatment of keratin wastes in Nigeria would benefit greatly from the findings of this research. To establish a cost-effective strategy for processing on a wide scale, further study is required to comprehend the mechanism of action of the degradation of feathers and other non-traditional protein sources.

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