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NUTRITIONAL QUALITY OF BACTERIAL COMPOSTED SUBSTRATES

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Abstract: **The study investigated the nutritional quality of bacterial composted substrates. The substrates namely: Cow hoof (1), chicken feather (2), egg membrane (3), hair waste (4) and cow horn (5)were subjected to bacterial composting for 42 days using *bacillus subtilis*. The substrates were collected and characterized physic-chemically and microbiologically. During the composting process, parameters such astemperature, total organic carbon (TOC), potassium, phosphorus, rate of degradation (ROD), pH, moisture content, ash, carbohydrate, crude protein, fibre, fat and bacterial counts; were monitored using standard methods. The results showed that physico-parameters measured were significantly different ($p<0.05$) among the substrates. Total organic carbon (%) before and after composting for substrates 1, 2, 3, 4 and 5 was 4.06 and 2.21, 12.79 and 0.51, 18.02 and 2.18, 20.34 and 2.10 and 9.01 and 3.80 respectively. Total protein (%) before and after composting was 62.7 and 58.5, 64.6 and 63.4, 1.60 and 1.53, 26.4 and 24.2 and 34.8 and 31.8, respectively.Total heterotrophic bacterial counts (10^3CFU/g) before and after composting was 6.4 and 5.48, 5.0 and 5.5, 6.0 and 4.38, 1.32 and 5.40 and 4.8 and 5.2, respectively. The ROD of substrate 2 was highest (96%), followed by substrate 4 (89.7%). The study suggests that substrate 2(chicken feather) has higher nutritional values and may be composted for fish and livestock feed formulation. This research is an important step toward the establishment of environmentally friendly technology for the treatment of keratin wastes in Nigeria.**

Keywords: **degradation, nutritional values, waste management and keratinase.**

1. INTRODUCTION

The management of solid wastes has become a serious challenge for many cities throughout the world as rural-urban migration and globalization continue to grow (Momodu *et al*., 2011).As a result of increasing livestock consumption, slaughterhouses produce large amounts of animal waste on a regular basis (Abdeshahian *et al*., 2016). Feather, cow hoof, and horn waste have all contributed to the daily increase in environmental pollution (Anbesaw, 2022). Due to its tenacious nature, animal waste, particularly feather, hoof, and horn, has become a major contaminant as a result of improper management.

Furthermore, they serve as breeding grounds for many salmonella and other pathogenic microorganisms, which release pollutants such as nitrous oxide, hydrogen sulfide, and heavy metals, all of which are harmful to human health and the environment (Ayilara *et al*., 2020). Poultry feathers, cow hooves, and horn are protein-rich waste products from the livestock processing industry, containing around 90% protein and high levels of amino acids such arginine, cystine, and threonine (Pfeuti, 2017). Conventional protein and the energy required to produce it have led to the high price of animal feed. As a result, it is vital to seek for alternative inexpensive protein sources for animal production, particularly from livestock waste, which is widely accessible.

Traditional techniques of processing, such as steam and pressure, as well as strong alkaline or acid, needed a substantial amount of energy and resulted in the loss of several important amino acids (Wang and Parsons, 1997). Therefore, biodegradation of livestock processing waste is a potential solution for developing a viable end product; it is one of the more cost-effective and ecologically safe recycling methods (Nnolim *et al*., 2020).As a consequence, it appears that investigating alternate methods is worthwhile. Several microorganisms, including several bacterial species (*Pseudomonas aeruginosa*, *Microbacterium sp., Bacillus licheniformis and B. pumilus)* 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 conductedto understand the biodegrading process of bacteria composting.

2. MATERIALS AND METHODS

2.1 Experimental design

The non-conventional protein sources used were cow hoof and horn, poultry feather, hair waste and egg membrane with *bacillus subtilis* as the bacteria inoculum in a randomized complete block designed (RCBD).

2.2 Selection and Collection of Non-Conventional Protein Sources

The various substrates were sourced within the University of Port Harcourt environment while the bacteria culture was collected from a private microbiology laboratory in Port Harcourt, Rivers State, Nigeria

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

Reactors	Substrates	Inoculum (10^6cfu/g)	Total solid (g)	Moisture content (g)	Dry solid(g)	Ash content(g)	Volatile solid(g)	Bacterial load X10^3cfu/g)
1	CHV	88	10	0.07	9.93	0.03	9.9	6.4
2	FeM	88	10	0.04	9.96	0.12	9.84	5.0
3	EM	88	10	0.05	9.95	0.27	9.68	6.0
4	HW	88	10	0.06	9.94	0.01	9.93	1.32
5	CH	88	10	0.12	9.88	0.13	9.75	4.8

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

2.3 Physico- chemical and microbiological analysis of compost

Physico-chemical parameters were determined as described by APHA, (1998), while bacterial load was determined using cultural methods as described by Selvankumar *et al.*, (2018).

2.4 Statistical analysis

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

3. RESULTS

3.1 Biological and physico-chemical parameters of the substrate

The result of the mean variation of biological and physico-chemical parameter of the substrates are shown in Table 2. Temperature of substrate 3 (29.79±0.04) was significantly higher than substrate 5 (29.66±0.04), 1 (29.62±0.04) and 4 (29.48±0.04) but was not different from substrate 2 (29.75±0.04). The percentage rate of degradation (ROD) in substrate 2(68.15±6.22), 3 (60.96±6.22) and 4 (62.13±6.22) were not significantly different from each other; but substrate 1 had the lowest degradability. Furthermore, the total nitrogen present in substrates 1(6.28±0.43) and 2 (5.60±0.43), was significantly higher compare to substrates 4 (3.02±0.43), 5 (3.99±0.43) and 2(0.19±0.43) respectively. Bacterial counts in each of the substrates revealed that there was a significant ($p<0.05$) difference in the composting ofthe substrates.

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

During weekly microbial decomposition of substrates, the temperature inside the bio-composter for cow hoof, chicken feather, egg membrane, hair waste and cow horn ranged from 29.5ºC to 29.9, 29.6 to 30 ºC 29.3 to 30.2 ºC, 29.7 to 29.9 ºC and 29.5 to 29.9 ºC respectively (figure 1). Values of pH ranged from 6.8 to 7.95, 7.3 to 8.0, 7.8 to 8.3, 7.0 to 8.0 and 6.7 to 7.8 for substrates 1 to 5 (figure 2). The total organic carbon content ranged from 2.21 to 4.06, 0.51 to 12.79, 2.18 to 18.02, 2.10 to 20.34 and 3.8 to 9.01 for substrates 1 to 5 respectively (figure 3). The rate of degradation ranged from 0 to 41.1%, 0 to 96.1%, 0 to 87.9%, 0 to 89.7% and 0 to 57.8% for substrates 1 to 5 respectively (figure 4.). The total nitrogen content ranged from 4.10 to 9.72 %, 1.68 to 10.24%, 0.03 to 0.35%, 1.50 to 5.41% and 1.42 to 6.48% for substrates 1 to 5 respectively (figure 5). Similarly, potassium concentration ranged from 6.8 to 7.40%, 1.3 to 1.60%, 0.09 to 0.19%, 0.27 to 0.34% and 1.3 to 1.41% for substrates 1 to 5 respectively(figure 6). Phosphorous content ranged from 6.2 to 7.85%, 8.05 to 8.50%, 0.11 to 0.13%, 0.18 to 0.20% and 5.82 to 6.20% (figure 7). Total bacterial counts ranged from 2.86 to 6.4 x 10^3cfu/g, 1.62 to 5.5 x 10^3cfu/g, 2.62 to 6.0 x 10^3cfu/g, 1.32 to 5.40 x 10^3cfu/g and 2.9 to 5.2 x 10^3cfu/g for substrates 1 to 5 respectively (figure 8).

Table 2: Mean biological and physico-chemical parameter monitored during bio-composting of non-conventional protein

Parameters	Substrates				
	1	2	3	4	5
Temp(^{O}C)	29.62±0.04^c	29.75±0.04^{ab}	29.79±0.04^a	29.48±0.04^d	29.66±0.04^{bc}
pH	7.35±0.07 ^{c}	7.61±0.07^b	8.06±0.07 ^{a}	7.42±0.07 ^{c}	7.41±0.07 ^{c}
TOC(%)	3.25±0.99^b	4.09±0.99 ^{b}	7.05±0.99^a	7.72±0.99^a	6.06±0.99^{ab}
ROD (%)	19.61±6.22^b	68.15±6.22^a	60.96±6.22^a	62.13±6.22^a	32.91±6.22^b
T-N(%)	6.28±0.43 ^{a}	5.60±0.43 ^{a}	0.19±0.43^c	3.02±0.43 ^{b}	3.99±0.43 ^{b}
K(%)	7.13±0.12 ^{a}	1.45±0.12^b	0.15±0.12 ^{c}	0.31±0.12^c	1.37±0.12^b
P(%)	7.20±0.16^b	8.17±0.16 ^{a}	0.13±0.16 ^{d}	0.20±0.16^d	6.09±0.16^c
THBC (X10^3cfu/g)	4.32±0.25 ^{a}	3.65±0.25 ^{a}	3.97±0.25 ^{a}	2.59±0.25 ^{b}	3.80±0.25 ^{a}

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, THBC= Total bacteria counts (X10^3cfu/g)(Substrates 1 to 5 cow hoof, chicken feather, egg membrane, hair waste and cow horn, respectively)

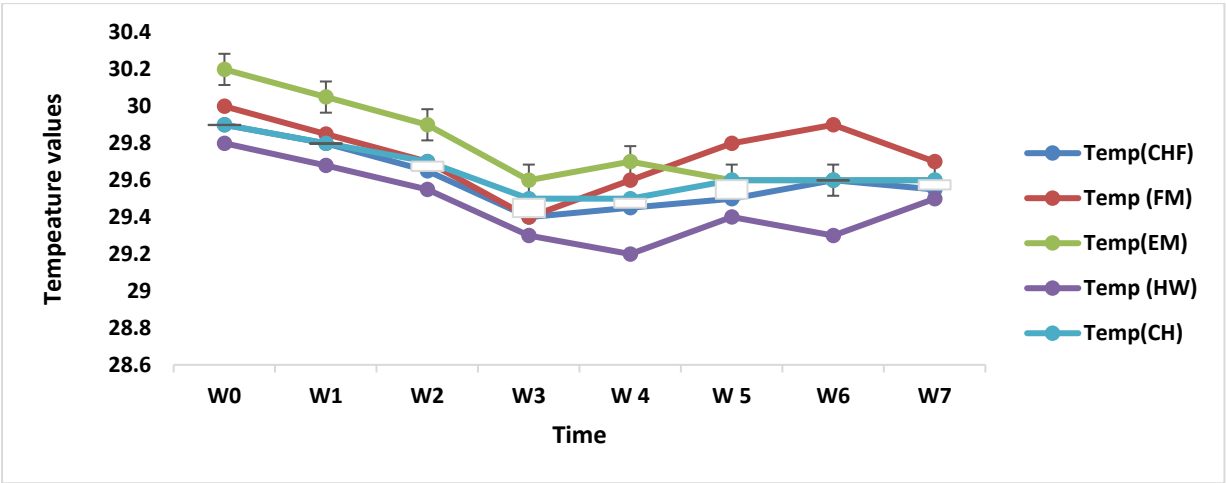

Figure 1: Changes in temperature during bacterial composting of substrates

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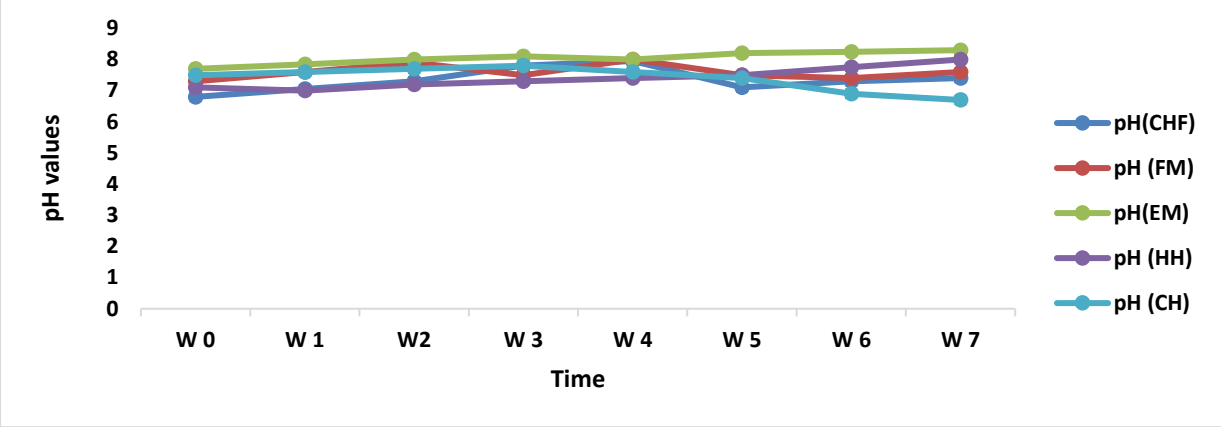

Figure 2: pH values using bacterial composting of substrates

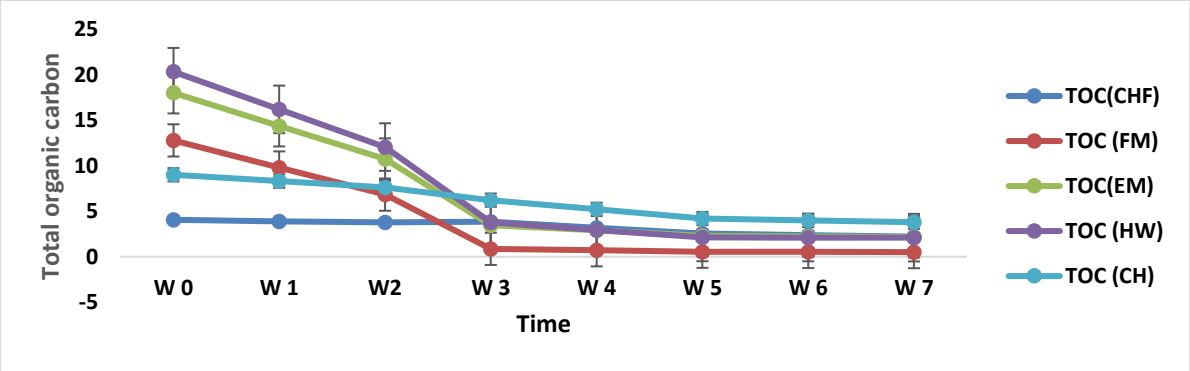

Figure 3: Total Organic Carbon values using bacterial composting substrates

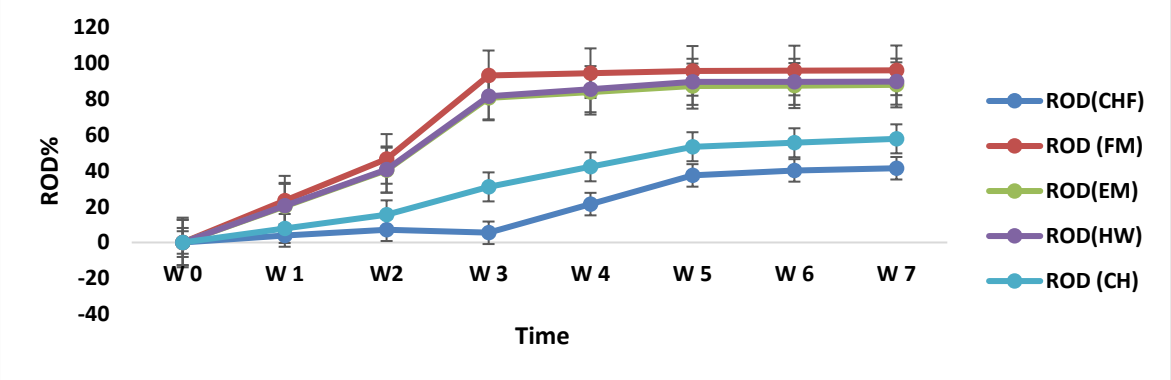

Figure 4: Rate of Degradation values during bacterial composting of substrates

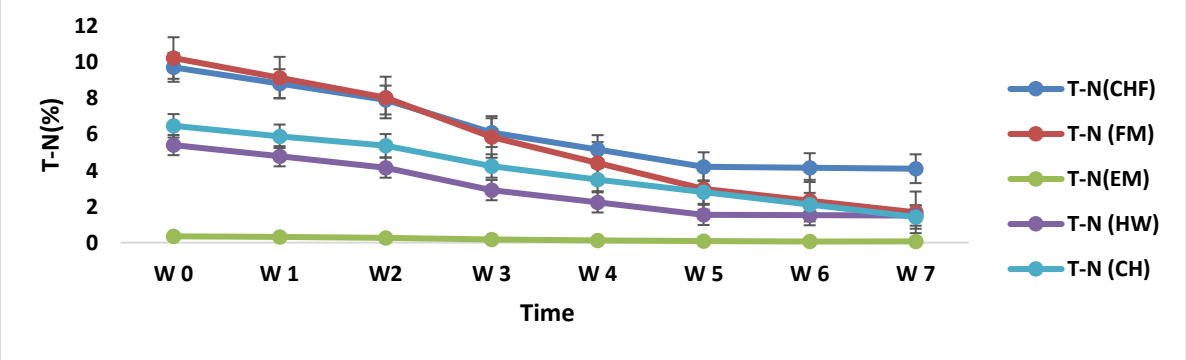

Figure 5: Total Nitrogen values during bacterial composting of substrates

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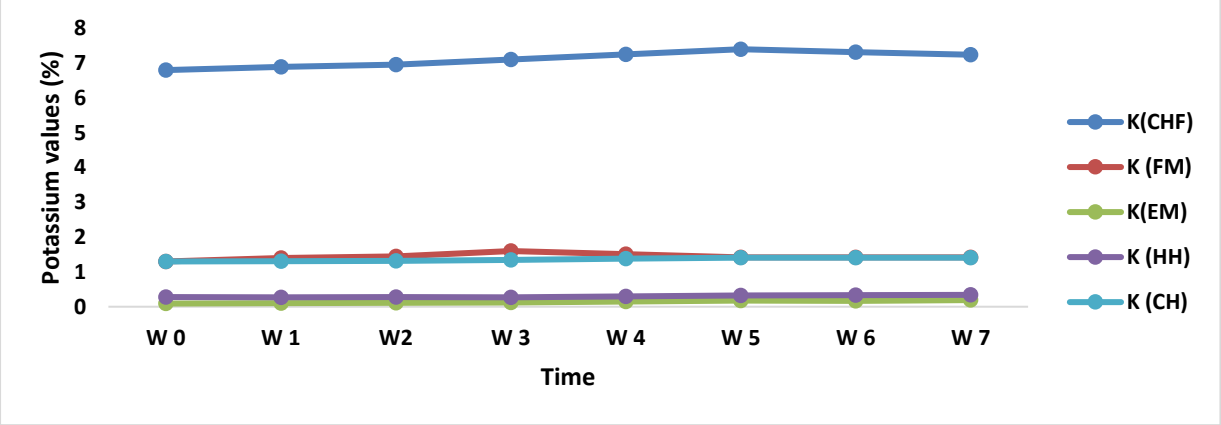

Figure 6: Potassium (K) values during bacterial composting of substrates

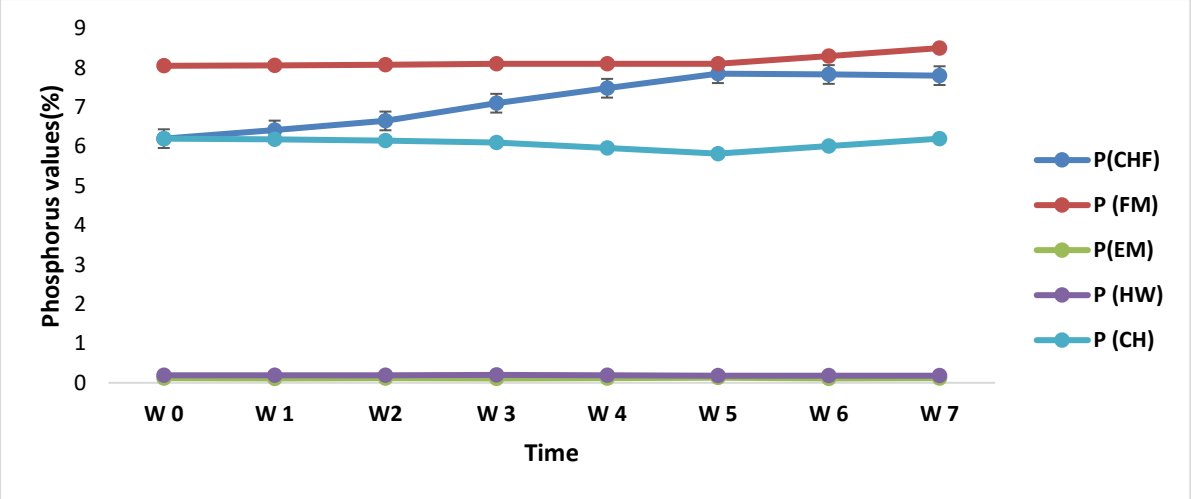

Figure 7: Phosphorus (P) values during bacterial composting of substrates

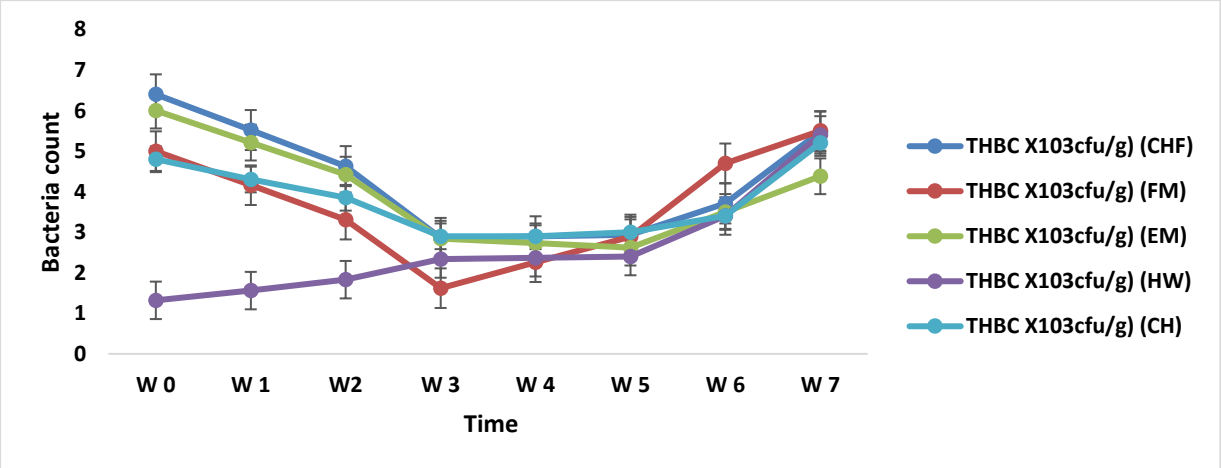

Figure 8: Bacterial counts during bacterial composting of substrates

3.3 Proximate composition monitored during bacteria composting of substrates

The results observed from the proximate composition monitored showed significant ($p<0.05$) difference. Moisture content in substrates 2 (66.68 ± 5.68^{a}), 3 (62.03 ± 5.68^{ab}) and 4(64.69 ± 5.68^{ab}) was significantly higher than substrates 1 (48.36 ± 5.68^{bc}) and 5 (41.47 ± 5.68^{c}). Ash content in substrates 3 (28.10 ± 0.94^{a}) and 5(29.50 ± 0.94^{a}) were significantly higher than substrates 2 (14.59 ± 0.94^{b}), 1(6.46 ± 0.94^{c}) and 4 (3.59 ± 0.94^{d}) respectively. Ether extract content in substrate 1 (6.97 ± 0.05^{a}) recorded

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the highest significant than substrates 2 (6.01±0.05[b]), 3 (1.26±0.05[c]), 4(1.02±0.05[d]) and 5(0.72±0.05[e]). Crude protein in substrate 2(63.97±0.19[a]) was significantly higher compared to all other substrates 1(60.21±0.19[b]), 5(32.94±0.19[c]), 4(24.95±0.19[d]). Carbohydrate content in substrate 3(60.74±0.73[a]) was higher compared to substrates 5 (29.27±0.73[b]), 1 (15.07±0.73[c]), 2 (8.44±0.73[d]) and 4(0.36±0.73[e]).

Table 3: Mean proximate composition of substrate monitored using Microbe during bio-composting.

Parameters%	Substrates				
	1	2	3	4	5
Moisture	**48.36**±5.68[bc]	**66.68**±5.68[a]	**62.03**±5.68[ab]	**64.69**±5.68[ab]	**41.47**±5.68[c]
Ash	**6.46**±0.94 [c]	**14.59**±0.94[b]	**28.10**±0.94[a]	**3.59**±0.94[d]	**29.50**±0.94[a]
Ether	**6.97**±0.05[a]	**6.01**±0.05[b]	**1.26**±0.05[c]	**1.02**±0.05[d]	**0.72**±0.05[e]
Crude protein	60.21±0.19[b]	**63.97**±0.19[a]	1.57±0.19[e]	24.95±0.19[d]	**32.94**±0.19[c]
Crude Fibre	**7.44**±0.10[b]	**1.23**±0.10[d]	**3.49**±0.10[c]	**57.69**±0.10[a]	**0.77**±0.10[e]
Carbohydrate	**15.07**±0.73[c]	**8.44**±0.73[d]	**60.74**±0.73[a]	**0.36**±0.73[e]	**29.27**±0.73[b]

Mean values (mean ± standard error) withdifferent superscript in the same row are significantly different (p<0.05).

3.4 Weekly proximate parameter monitored during bio-composting of substrates

Moisture content ranged from 3.9, 4.9, 5.9, 11.8 and 6.9% to 62.5, 90, 85.5, 85.5 and 55.5% for substrates 1 to 5 respectively(Figure 9). Ash content range from 2.5 to 10.4%, 11.5 to 16.5%, 26.5 to 29.5%, 0.89 to 4.8 and 12.5 to 36.8% (figure 10), The ether extract range from 6.5 to 6.9%, 5.6 to 6.5%, 1.20 to 1.3%, 0.94 to 1.18% and 0.50 to 1.12% (figure 11). Crude protein rangedfrom 58.5 to 62.7%, 63.4 to 64.6%, 1.53 to 1.60%, 24.2 to 26.4% and 31.8 to 34.8% for substrates 1 to 5 respectively (figure 12). Crude fibre content ranged from 6.90 to 8.42, 1.16 to 1.28, 3.16 to 4.26, 56 to 59.1 and 0.73 to 0.86 (figure 13).The carbohydrate content ranged from 13.80 to 15.98, 7.56 to 10.22, 60.04 to 61.44, 0.24 to 0.63 and 23.42 to 43.74 for substrates 1 to 5 respectively (figure 14).

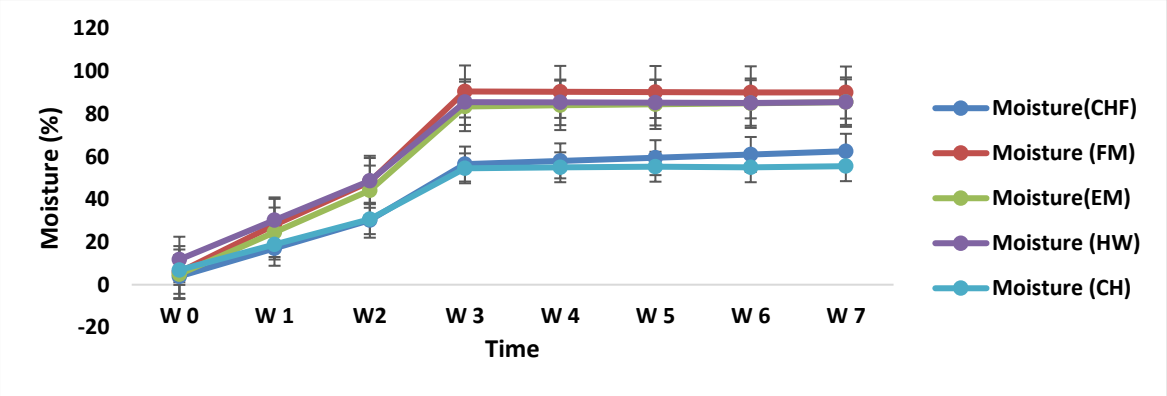

Figure 9: Moisture content monitored during bacterial composting of substrates

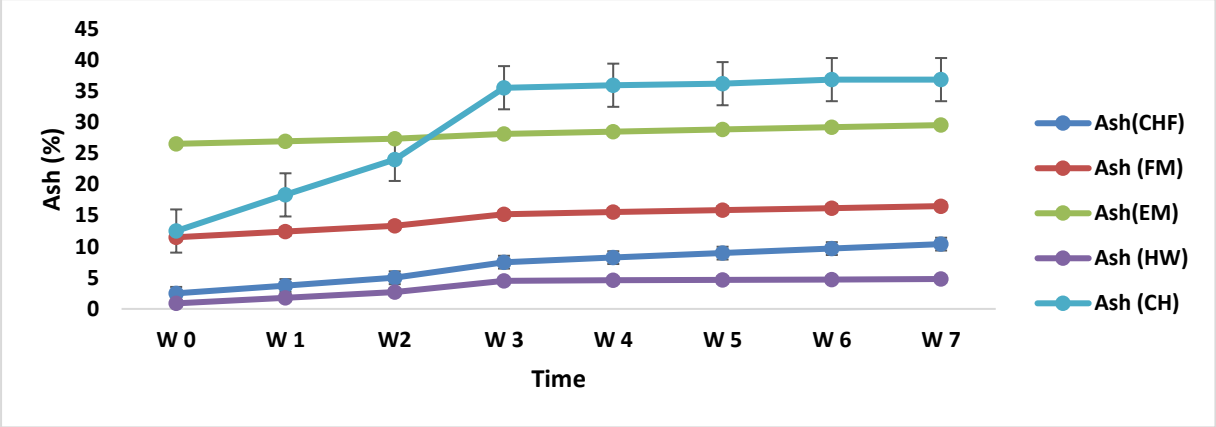

Figure 10: Ash content monitored during bacterial composting of substrates

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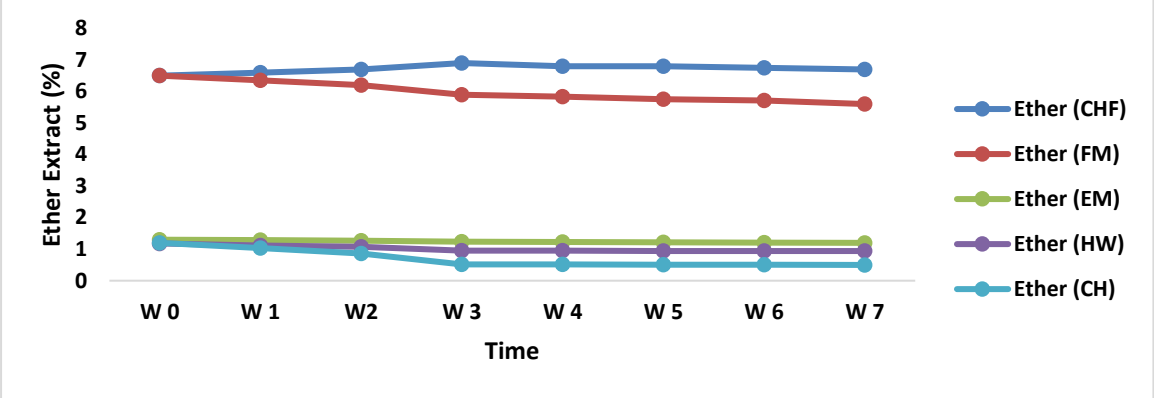

Figure 11: Ether extract content monitored during bacterial composting of substrates

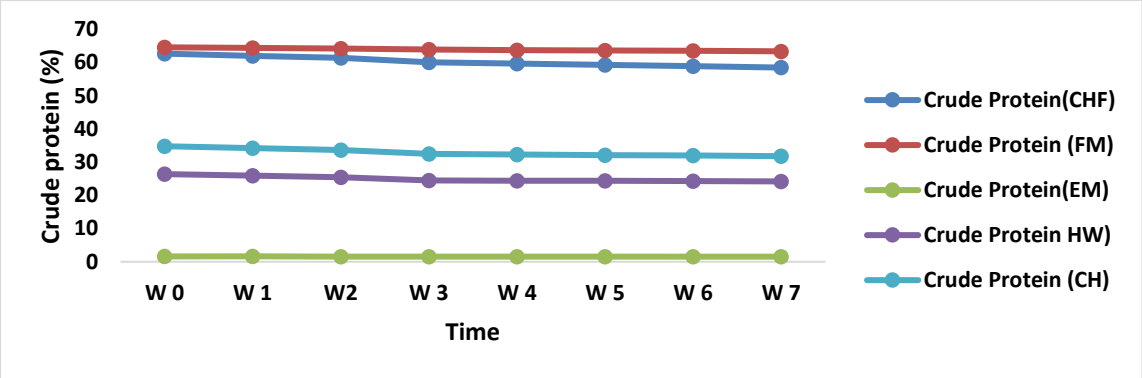

Figure 12: Crude protein content monitored during bacterial composting of substrates

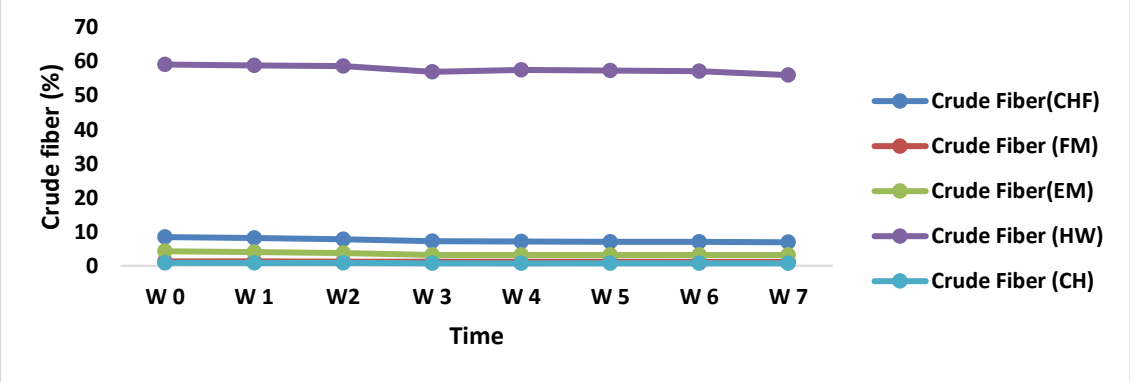

Figure 13: Crude fiber content monitored during bacterial composting of substrates

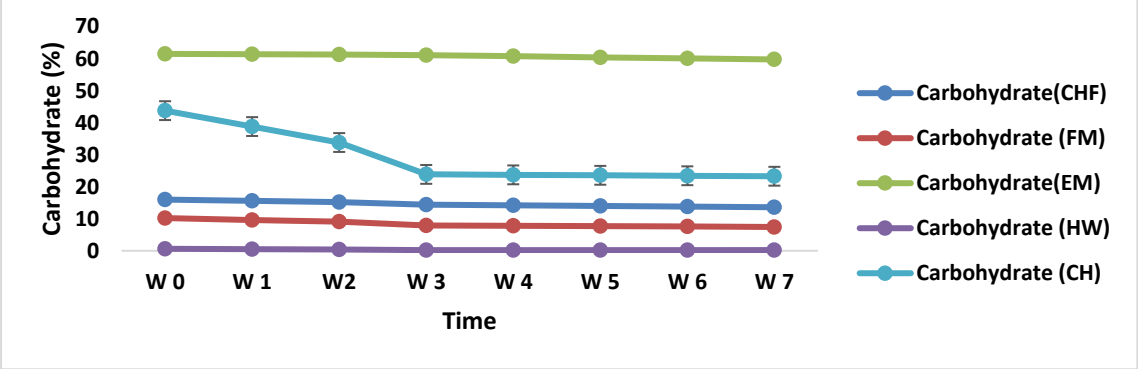

Figure 14: Carbohydrate content monitored during bacterial composting of substrates

4. DISCUSSION

The temperature range of substrates was elevated during the composting period. This could be as a result of aerobic process, heat increase and heat retention capacity of the bio-composter because they are made of plastic which are poor conductor of heat, water vapor and the release of CO_2 during decomposition process. Although some were higher than others like substrates 3 and 2 this might be due to the particle size of the substrates. Similar temperature differences were discovered (Lyndall *et al.*, 2004) during keratin degradation and composting heaps for the creation of environmentally friendly bio-fertilizers, Sekar *et al*., (2015) had 48.9 ^oC in their studies. During composting, the pH is usually increased for faster decomposition. This was not in line with Hachicha *et al*., (2008) who reported neutral pH values during the processing period, which is good compost recommended range (6.90 to 8.65) for feather compost. During week 1 to 4 composting process, the pH value increased and decreased from weeks 4 to 7 for all substrates apart from substrate 3. Ignatova *et al.* (1999) found that it may be due to digestion of keratinaceous waste. Bacteria produce organic acid and don't perform well in a reduce pH because the acid will kill them and reduce their performance. The production of CO_2 from organic acids and the loss of nitrogen can also be attributed (Lugtenberg *et al*., 2013).

The total organic carbon reduced significantly in all substrates during microbial composting, although values in substrate 5 were minimal compared to other substrates. However during the degradation process, total organic carbon content reduced. It's varied from 0.51 to 20.34 for bacterial composting. This result is difference from these obtained by (Sekar *et al.*, 2015) who reported a range of 11.56 to 41.34 % in all the samples. There was reduction in percentage rate of degradation. This showed that the microbes were able to breakdown the substrates. Some substrates degrade faster than others depending on their physical properties. The total nitrogen was highest in substrates 1 and 2 whereas substrate 3 had the lowest value. Similar result was observed by (Sekar*et al*., 2015) with variation of 0.38 to 1.84 %. The decrease in the nitrogen content during composting might be produced by slow degradation of organic substrate which contains amino sugars and proteins (Mondini *et al*., 2008).

Potassium in substrate 1 was higher compare to other substrates. The weekly range of potassium concentration varied from 0.09 to 7.40. This result was not in line with (Sekar*et al*., 2015) who observed a variation from 0.65 to 1.97%. Although, they had similar potassium increases during composting period. This might be due to an increase of potassium in the compost which is often due to degradation of organic cellular components.Phosphorous total concentration in substrate 2 was highest for bacteria. The weekly concentration varied from 0.11 to 8.56. This was not in line with (Sekar*et al*., 2015) that used (*Bacillus subtilis*FDS15) to compost feather and recorded a range of 0.11.to 0.47. Janakiram and Sridevi, (2010) reported that total phosphorous content increased gradually during the process of composting and decreased with humification of water solubility of phosphorous.The total bacterial count during the composting process was between the ranges of 1.32 to 5.52X10^3cfu/g to 2.06 to 6.7 X10^3cfu/g for bacteria. There was reduction of bacteria counts from week 1 to 4. However 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. There was a continuous increase of microbial count for hair waste.

The moisture level of the composting mix is a key environmental factor that creates a suitable environment for the transfer of dissolved nutrients required for microorganisms' metabolic and physiological processes (Janakiram and Sridevi, (2010). Compost moisture was observed to be increasing throughout the composting process; it might be as a result of the addition of water to the substrates to aid degradation and the microbial degrading process. Sekar *et al*. (2015) noted moisture loss during their experiment using bacteria on feather meal. Hayashida *et al.* (1988) reported similar findings on the removal of water from the starting material. At the end of the composting process, moisture content was increase to 93%. There was a continuous increase in ash content throughout the composting period across all substrate. Ether extract content was highest in substrate 1 than the other substrates. The ether content range during composting was from 0.50 to 6.9. There was also a corresponding decrease of ether content of substrate during the composting period.

Crude protein was highest in substrate 2, although substrate 3 had the lowest protein content. The range of crude protein degradation using bacteria *spp* was from1.53 to 64.6%.There was a decrease of crude protein during the composting period. This might be as result of the carbon dioxide release during aerobic bio-composting and breaking of the bond by the microorganism during the degradation process (Gupta *et al*., 2019).There was a decrease of crude fibre during the composting period. There was also a decrease of carbohydrate content throughout the composting period but feather meal recorded an increase in week 7. However using bacteria to decompose, feather meal had a decrease of carbohydrate content in week 7.

5. CONCLUSION

The study revealed that composting with bacteria resulted in the maximum degradability after the 4th to 7thweek of composting. This research is an important step toward the establishment of environmentally friendly technology for the treatment of keratin wastes in Nigeria. We are convinced that the microbial process of degradation can be used for feedstuff preparation. More research is needed to understand the mechanism of action of feather degradation and other non-conventional protein sources utilized to develop an economic approach for large-scale processing.

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