

Proliferation of Microorganisms in Acidic Fermentation of *Elaeis guineensis* L. Waste

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Abstract

An investigation into the type of fermentation oil palm fruit waste undergoes and the probable microorganisms involved within a short period was carried out using simple fermenter. The temperature was determined using mercury thermometer, the pH of the medium was monitored with calibrated pH meter and the titratable acidity was determined using standard technique. The microbial profile of the medium was also evaluated using standard procedures. The highest temperature value was observed at day 0 (32.65°C) and the lowest at day 1 (29.50°C). The pH values of the fermentation oil palm fruit waste ranged between 4.15 - 4.60. The highest pH value was obtained at day 3 of the fermentation which was 4.60. The titratable acidity showed variation from day 0 - 2 and then with a continuous decrease till day 5. The least titratable acidity was obtained at day 5 (0.03) and the highest at day 2 (0.77). Bacteria load decreases from 1.0×10^8 - 1.6×10^7 cfu/ml, while the fungi population increases from day 0 to day 5 of the fermentation period (1×10^3 - 2×10^4 sfu/ml). Bacterial isolates obtained were *Micrococcus luteus*, *Proteus vulgaris*, *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus* while the fungal isolates obtained were *Aspergillus niger*, *Neurospora crassa*, *Brachysporium* spp, and *Saccharomyces cerevisiae*. It can be concluded that oil palm waste fermentation is an acidic fermentation that involved mesophiles microbes. And with these, several tons of oil palm epicarp waste can be optimally fermented (though with further research) and used for other purposes thereby reducing environmental pollution that would have resulted leaving this oil palm fruit waste in the environment.

Keywords

Elaeis guineensis L., Fermentation, Microorganism, Proliferation, Waste

1. Introduction

Solid substrate fermentation has been exploited for the production of value added products namely antibiotics,

alkaloids, plant growth factors, bio-fuels, enzymes, organic acid and biological detoxification of agro-industrial residues, nutritional enrichment, pulping, bio-pharmaceuticals product [1]. Fermentation is one of the oldest applied biotechnological methods having been employed in food processing for many years [2]. Fermentation either naturally or with selected microbial inoculums has also been extensively used to enhance the nutrient potential of oil palm and its by-products for human use, and contribute significantly to food security. It also enhances micro content bioavailability and aids degrading anti-nutritional factors [3]. Microbial growth in agricultural and food processing wastes offers the potential for additional food and feed of a wide variety for domestic and farm animals. The microbes can produce many times as much protein per area than slow growing plants and animals. Thus high fat content and low protein crops like oil palm waste can be enhanced nutritionally through microbial conversion.

The oil palms comprise two species of the Arecaceae or palm family. They are used in commercial agriculture in the production of palm oil. The African oil palm *Elaeis guineensis* is native to West Africa occurring between Angola and Gambia, while the American Oil Palm *Elaeis oleifera* is native to tropical Central America and South America. Each palm fruit is made up of an oily, fleshy outer layer, with a single seed (the palm kernel), also rich in oil. Oil is extracted from both the pulp of the fruit (palm oil, edible oil) and the kernel (palm kernel oil, used in foods and for soap manufacture). After oil extraction, the remaining shaft is thrown away as waste. This can become nuisance to the environment, if not properly disposed. Palm oil increasing use in the commercial food industry though buoyed by its cheaper pricing, high oxidative stability of the refined product and high levels of natural antioxidants [4], has also lead to increase in the amount of oil palm waste generated.

The oil palm by-product is acceptable to ruminant at low level inclusion in the diet. Crude protein and crude fiber digestibility decrease when the level of inclusion exceeds 25% - 30%. The fiber can be dried and pelletized to overcome the problem of poor keeping quality and bulkiness. Fermentation can be employed to improve the protein content and generally the digestibility of this waste. This research is sought to investigate the type of fermentation oil palm fruit waste undergoes and the probable microorganisms involved within a short period. Thereby, encouraging its removal from the environment, to be use as inclusion in animal diets.

2. Materials and Methods

2.1. Collection of Sample

Fresh palm oil waste was obtained from Nigerian Institute For Oil Palm Research (NIFOR) near Benin City, Edo state (6°30'N 6°00'E). The types of fruit processed are mixture of *Dura* and *Tenera* varieties.

2.2. Solid State Fermentation

Dried sample of oil palm waste was weighed (about 500 g) and rapped with foil paper and was sterilized in the autoclave for 15 minutes at 121°C, after which the oil palm waste was aseptically transferred into a sterile bowl (fermenter), 500 mls of sterile water was aseptically added and then allowed to ferment for 5 days. Sample was taken for titratable acidic daily while the pH and the temperature were also determined daily in the fermenter using pH meter and mercury thermometer respectively. The microbial loads were monitored daily. Isolation of bacteria and fungi from the fermenting palm waste was carried out on the fifth day.

2.3. Physicochemical Parameters

The physicochemical parameters measured were: pH, temperature, and titratable acidity determination [5].

2.4. Enumeration of Fungi and Bacteria Counts

Spore/colony counting was carried out by counting the number of visible spores/colonies that appeared on the agar plates. Nutrient agar was used for bacteria culture while potatoes dextrose agar was used for fungi culturing. Calculation of spore/colony forming unit (sfu/cfu) per ml for fungi and bacteria were based on the volume of the sample used.

2.5. Cultural and Microscopic Characteristics of Fungi

The fungi were isolated on potatoes dextrose agar and each colony was identified using visible observation and

microscope at low power magnification ($\times 40$), the parameters such as colony colour, characteristics of the submerged hyphae rhizoid, spiral or regular and characteristic shape of mature fruiting bodies were all observed. Microscopic characterization involved transferring a small piece of mycelium free of medium using a sterile inoculating loop unto a clean glass slide containing a drop of cotton blue-in-lactophenol and the mycelium was spread properly. The preparation was covered with a clean grease free cover slip and observed under medium power ($\times 100$). The observations made were used in identifying the fungi organism [6].

2.6. Biochemical and Morphological Identification of Bacteria Isolates

Bacteria were isolated using nutrient agar and the individual colonies were identified by morphological and biochemical techniques using methods described by [7] [8].

3. Results

A total of 5 bacterial isolates were obtained based on their morphological and biochemical properties for the bacteria and a total of 4 fungal isolates were obtained. The bacteria were: *Micrococcus leteus*, *Proteus vulgaris*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*. And fungi were: *Aspergillus niger*, *Neurospora crassa*, *Brachysporium* spp, and *Saccharomyces cerevisiae*. **Table 1** shows the microbial population of oil palm waste during fermentation. Oil palm wastes microbial load decreases from 1.0×10^8 - 1.6×10^7 cfu/ml. While the fungi growth increases from day 0 to day 5 of the fermentation period (1×10^3 - 2×10^4 sfu/ml).

3.1. pH Values Observed during Fermentation

The pH values of the fermentation process were below the neutral pH of 7.00. It ranged between 4.15 - 4.60 (**Figure 1**). The highest pH value was obtained at day 3 of the fermentation which was 4.60.

Table 1. Microbial population of oil palm wastes during liquid state fermentation.

| Days | Fungi sfu/ml | Bacteria cfu/ml |
|-------|-----------------|-------------------|
| Day 0 | 1×10^3 | 1.0×10^8 |
| Day 1 | - | 2.1×10^7 |
| Day 2 | 2×10^3 | 2.8×10^7 |
| Day 3 | 2×10^4 | 1.6×10^7 |
| Day 4 | - | 1.6×10^7 |
| Day 5 | 2×10^4 | 2.3×10^8 |

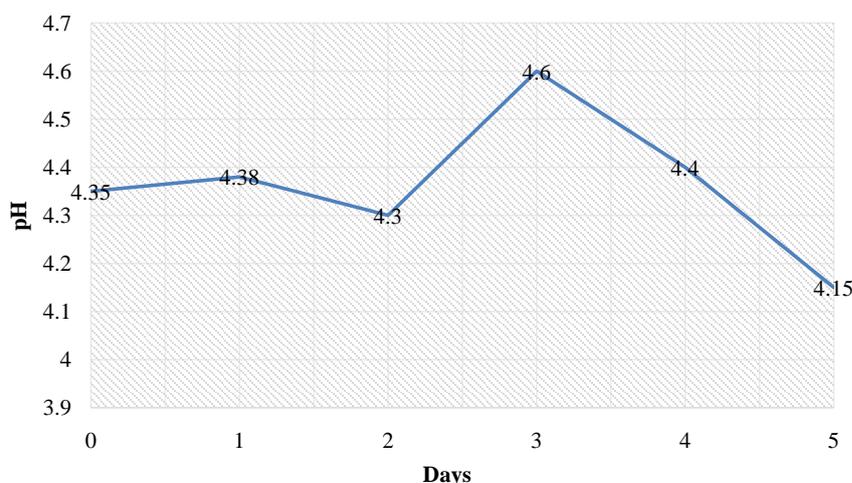


Figure 1. pH of fermented oil palm waste.

3.2. Acidity of Oil Palm Waste Observed during Fermentation

The titratable acidity showed variation from day 0 - 2 and then with a continuous decrease till day 5. The least titratable acidity was obtained at day 5 with 0.03 and the highest at day 2 with 0.77 (Figure 2).

3.3. Temperature Observed during Fermentation

Figure 3 show the daily temperature of oil palm waste during the fermentation. The highest value was obtained at day 0 with 32.65°C and the lowest at day1 with 29.50°C.

4. Discussion

Table 1 shows the microbial population of oil palm waste during fermentation. Oil palm wastes bacterial load ranged from 1.0×10^8 - 1.6×10^7 cfu/ml. While the fungi growth ranged from 1×10^3 - 2×10^4 sfu/ml. These show there were more bacterial activities in the medium than fungal activities [9].

The microorganisms isolated were probably acidophiles either strict acidophile or facultative acidophiles. Since the pH of the fermentation process was acidic throughout the five days period, their survival show they were acid tolerant microbes. *Bacillus* spp, *Aspergillus niger* and *Saccharomyces cerevisiae* are common fermentative microbes [10]-[16], their isolation in this study further confirms this. This further shows their versatility

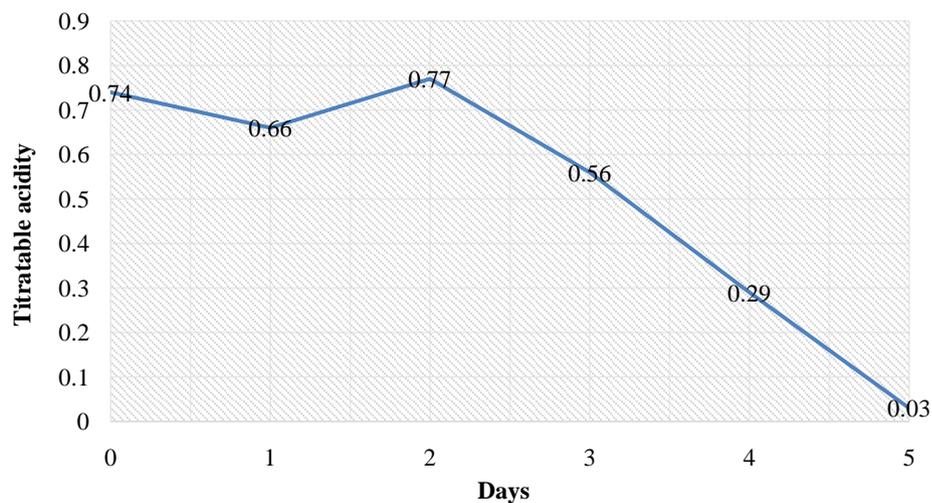


Figure 2. Titratable acidity of fermented oil palm waste.

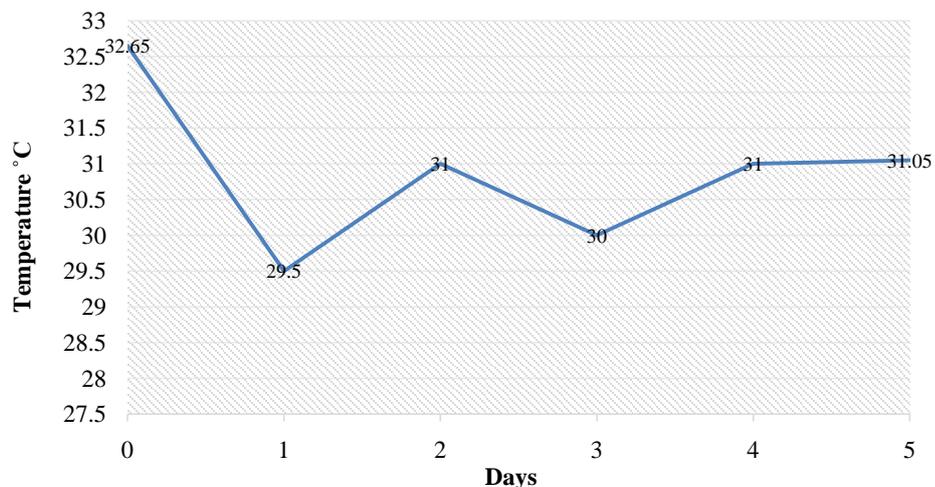


Figure 3. Temperatures of the fermentation process of oil palm waste.

in nutrient usage, substrate usage and their fermentative involvement [15] [17] [18].

The fermentation medium was acidic throughout the period in view which was evident in the values of the pH (Figure 1), though varied from day to day. This might be due to the microbial activities in the medium [19]. Acidic products or by-products must have been released to the medium to have kept the pH values low. This fermentation probably resulted from acidic pathway(s), further research can confirm this. The 3rd day had the highest pH value (4.60) this probably was the highest period of release of acidic products into the medium. At the same time, the titratable acidity followed similar trend as the pH values (Figure 2), showing close relationship between the two parameters. This agreed with the report of [20] that variation in titratable acidity and pH is a function of the fermentative activities of microorganisms present in a product and the length of fermentation.

The temperature values (Figure 3) which ranged from 29.5°C - 32.5°C revealed the fermentation process occurred under moderate temperatures. This shows the fermentation of oil palm fruit waste was not a high heat producing process.

5. Conclusion

In conclusion, several tons of oil palm pericarp waste can be properly or optimally fermented with insight this research has thrown into the fermentation of oil palm waste. Fermentation had been known to improved food qualities therefore fermented oil palm waste may be used to improve farm animals' feeds when incorporated. This can in a way reduce the cost spent by farmers in producing animal feeds and as well reduce environmental pollution that would have resulted leaving this oil palm fruit waste in the environment.

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