INFLUENCE OF FERMENTATION ON THE NUTRITIONAL CONTENT OF COCOA POD (*Theobroma cacao* L)

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**Abstract**

Studies were carried out to determine the effect of fermentation on cocoa pod after removing the bean. The pods were collected from Oba Market in Akure, Ondo State and sun dried. Part of it was fermented for 96 hours during which pH, temperature; total titratable acidity, microbial population, isolation and identification of microorganisms were carried out and then dried for proximate analysis. The unfermented sample was also subjected to proximate analysis. The total mean bacterial count was between $3.3 \times 10^5$ and $6.0 \times 10^5$ cfu/ml while the mean fungal count ranged from $1.8 \times 10^5$ to $4.1 \times 10^5$ sfu/ml. A total of nine organisms comprising of both bacteria and fungi were isolated. The bacterial isolates were five and they include *Bacillus subtilis*, *Leuconostoc mesenteroides*, *Lactobacillus delbrueckii*, *Proteus vulgaris* and *Staphylococcus aureus*. Whereas, four fungi were isolated; *Aspergillus niger*, *Aspergillus flavus*, *Mucor mucedo*, *Penicillium notatum*. The pH of the fermenting cocoa pod ranges from 6.5 to 4.6. The total titratable acidity ranges from 0.5 to 2.7 and temperature from 24 to 32°C. The proximate of unfermented and fermented cocoa pod are as follows: moisture content (9.57, 12.39%), ash content (3.74, 4.02%), fat content (3.71, 2.55%), crude fiber (15.55, 7.50%), protein (6.44, 6.11%), carbohydrate (60.71, 68.02%). This result reveals that fermentation has a positive effect on some nutrients of cocoa pod by considerably increasing its nutritional content and has brought about a decrease in some of the nutrients.

Key words: Cocoa pod, fermentation, influence, nutritional content.

**INTRODUCTION**

Tropical New World tree (*Theobroma cacao*), with varieties like Criollo and Forastero, is of the chocolate family (*Sterculiaceae*). Its seeds, after fermentation and roasting, yield cocoa and chocolate. The tree produces pods that contain about 40 cocoa beans surrounded by a sweet tasting mucilaginous pulp. In Nigeria, it is a plant grown under bean remnant forest by mostly small holders accounting for about 90 percent of aggregate hectarage (Opeke, 1997) but who employ cheap labour to achieve harvest. Upon harvest the pod becomes a source of diverse utility. However, the huge volume of the cocoa pod husk, in Nigeria calls for a maximum attention aimed at increasing the utility value. Concentrating on this importance further invites the need to subject many component parts to as much scrutiny as possible.
As at date, in Africa the cocoa pod husk is used as an ingredient in the diet of growing pigs (Oddoye et al., 2010), as starter cockerels (Olubamiwa et al., 2002) as a fertilizer (Agbeniyi et al., 2011) and as an effective replacement of dried guinea grass in sheep ration (Adomako et al., 1999). In 1999, Tuah et al reported a promising outcome of a group of sheep fed on various proportions of cocoa pod husk. This is particularly interesting because there is no fear of difficulty in degrading the husk, which are rich in lignin, in these ruminants. This property is recognized in Indonesia as several thousand tons of the husks are used in cattle feedlots regularly. The tannin content is a potent antifungal (Brownlee et al., 1990)

The husk is known to have medicinal properties having been traditionally applied to treat the pains of pregnancy, fevers, and coughs. Theobromine relaxes the smooth muscle in the digestive tract. It can also be used to combat fatigue and protect the cardiovascular muscles. This research was at investigating: the impact of fermentation of the cocoa pod on the proximate analysis and microbial load.

MATERIALS AND METHODS

Sample collection

Ripe cocoa pods were purchased in Oja Oba in Akure, the capital of Ondo State. The samples were collected in sterile polythene bags and taken immediately to the laboratory. The colour of ripe pods varied from yellowish green to fully yellow husk. The ripe cocoa pods were then split open with a sharp knife and the cocoa beans were removed.

Fermentation of cocoa pod

Fermentation of the cocoa pod samples was done using the submerged fermentation method. The cocoa pods were then cut into bits, divided into four parts of 100g each and then measured into four different labeled containers containing 200ml of water for fermentation. Samples were taken on first, second, third and fourth day for analyses. Unfermented cocoa pod samples and the first - fourth day fermented cocoa pod samples were all sun-dried. The dried cocoa pods were ground using mortar and pestle into powder as samples for determination of moisture content, crude fiber, fat content, ash content, protein content and carbohydrate content in the two samples.

Microbial Isolation

For microbial isolation, the streak plate method was used. A loopful of $10^4$ dilution of the sample was taken and streaked on both nutrient agar and potato dextrose agar plates for bacterial and fungal isolation respectively. The plates were then incubated at appropriate temperatures and then observed.

Characterization and microscopy of fungal isolates

Colonial morphology and microscopic examination of the various fungal isolates from the stock culture were carried out to determine the reproductive and vegetative structures. Some of the colonial characteristics observed on PDA medium which include colour of the colony, rate of growth, production of pigment released into the medium, colour of reverse side of the plate.

For microscopic examination, a portion of the mycelium was picked using a sterilized inoculating needle. This was teased with the inoculating needle and covered with a cover slide. It was then observed under X40 objective of a microscope.

Characterization and identification of Bacterial isolates

The bacterial isolates were characterized with respect to their colonial characteristics such as colour, colony, size, elevation, surface texture, consistency and light penetration. Biochemical characterization was also performed according to the method of Fawole and Oso (2001).

Proximate analysis

The proximate composition of the nutrients of unfermented and fermented cocoa pods, namely moisture content, ash content, crude fibre content, fat content, protein content and carbohydrate was analyzed and recorded by the AOAC method.
Determination of total titratable acidity (TTA)
The titratable acidity of the fermenting sample of cocoa pod was calculated by measuring 10ml of the sample solution and few drops of phenolphthalein was added. This was titrated against 0.1M sodium hydroxide (NaOH) solution on a daily basis and the titre values in milliliters were read from the burette. (Usoro et al., 1982)

RESULTS
Microbial load of cocoa pod in the course of fermentation
The total mean bacterial counts ranges from $3.3 \times 10^5$ to $6.0 \times 10^5$ cfu/ml while the mean fungal count ranges from $1.8 \times 10^5$ to $4.1 \times 10^5$ sfu/ml. The lowest microbial load was recorded on the first day of fermentation while the highest microbial load was observed on the second day of fermentation. This result is shown in Table 1.

Table 1: Bacterial and Fungal count of fermented cocoa pods

<table>
<thead>
<tr>
<th>Period (hours)</th>
<th>Bacteria (cfu/ml)</th>
<th>Fungi (sfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>$3.3 \times 10^5$</td>
<td>$3.5 \times 10^5$</td>
</tr>
<tr>
<td>48</td>
<td>$6.0 \times 10^5$</td>
<td>$4.1 \times 10^5$</td>
</tr>
<tr>
<td>72</td>
<td>$5.1 \times 10^5$</td>
<td>$3.0 \times 10^5$</td>
</tr>
<tr>
<td>96</td>
<td>$3.8 \times 10^5$</td>
<td>$1.8 \times 10^5$</td>
</tr>
</tbody>
</table>

Table 2: Proximate composition of unfermented and fermented cocoa pod

<table>
<thead>
<tr>
<th>Proximate composition (%)</th>
<th>Unfermented pod</th>
<th>Fermented pod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content</td>
<td>9.57</td>
<td>12.39</td>
</tr>
<tr>
<td>Ash content</td>
<td>3.74</td>
<td>4.02</td>
</tr>
<tr>
<td>Crude fiber content</td>
<td>15.55</td>
<td>7.50</td>
</tr>
<tr>
<td>Fat content</td>
<td>3.71</td>
<td>2.55</td>
</tr>
<tr>
<td>Protein content</td>
<td>6.44</td>
<td>6.11</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>60.71</td>
<td>68.02</td>
</tr>
</tbody>
</table>

Figure 1: pH and Total Titratable acidity during fermentation of Cocoa pod

Keys:

TTA – Total Titratable Acidity
Aspergillus flavus, Mucor mucado, and Penicillium notatum were isolated from the fermented cocoa pod. The pH range of the fermented cocoa pod was from 4.6 to 6.5 (Figure 1). An increase in pH was observed, possibly due to evaporation of volatile acids like acetic acid. The temperature was observed to increase by 2°C after each day. Temperature for 0 hour to 72 hours of fermentation were 26°C, 28°C, 30°C, and 32°C respectively. The gradual increase in temperature of the fermenting cocoa pod from the first to the last day is probably due to the anaerobic fermentation which lead to the depletion of oxygen.

There was a decrease in the pH between 0 hour and 72 hours of fermentation and a rise in pH on the last day of fermentation with a corresponding increase in the values of TTA of fermented cocoa pod between 0 hour and 72 hours of fermentation and a decrease in TTA value of fermented cocoa pod on the last day of fermentation (Figure 1). The pHs for 0 hour to 72 hours of fermentation are 6.5, 5.9, 5.2, and 4.6 respectively. The observed consistent decrease in pH within the first 3 days of fermentation was probably due to the diffusion of organic acids predominantly acetic acid into the beans produced by acetic acid bacteria in the pulp. While towards the end of fermentation, an increase in pH was observed, possibly due to evaporation of volatile acids like acetic acid.

The temperature of the fermenting Cocoa pods was measured and represented in figure 2. The temperature was observed to increase by 2°C after each day. Temperature for 0 hour fermentation was 24°C, 24°C, 48°C, 72°C, and 96°C respectively. The gradual increase in temperature of the fermenting cocoa pod from the first to the last day is probably due to the anaerobic fermentation which lead to the depletion of oxygen.

Proximate analysis of the fermented and unfermented pods shown in Table 2 revealed that moisture content was increased in the fermented cocoa pod (9.57% in unfermented pod and 12.39% in fermented pods). This may be because microorganisms involved in the fermentation did not utilize much water during the fermentation process.

The ash content of the fermented sample (4.02%) was greater than the ash content of the unfermented sample (3.74%). This result agrees with the work of Adamafio et al. (2011) which says that the ash content of the fermented cocoa pod was surprisingly higher than that of the unfermented one and also said that this may be due to the fact that microorganisms helped to breakdown or convert some higher molecular weight minerals into more minerals with lower molecular weight.
The protein content (table 2) of the unfermented cocoa pod is more than that of the fermented cocoa beans. This is understandable since the microorganisms involved in the fermentation of the beans will also utilize proteins and break them down to amino acids that will be useful for their metabolism (as their nitrogen source).

The fat content of the fermented pod is higher than that of the unfermented pod.

The crude fibre content of the unfermented pod was greater than that of the fermented pod probably because of its usage by the microorganisms as energy source.

The carbohydrate content of the fermented pod surprisingly increased from 60.71% (for the unfermented pod) to 68.02%. This might be because the organisms did not use carbohydrate as energy source thereby not causing depletion of the carbohydrate.

In general, the study shows that fermentation caused an increase on half of the nutrient content of cocoa pod (moisture content, ash content and carbohydrate) and also has a negative effect on half of the nutrients.

CONCLUSION AND RECOMMENDATION

CONCLUSION

The result of this study reveals that fermentation does not always bring about an increase in nutritional content i.e. fermentation has a positive effect on some nutrients while it has a negative effect on some nutrients.

RECOMMENDATION

Despite the reduction in some nutrient of cocoa pod caused by fermentation, I still strongly recommend its use as animal feed supplement since there is an appreciable increase in the carbohydrate, moisture and ash content after fermentation which could serve as a very good source of energy for livestock.

REFERENCES


