Effect of Fermentation on Biochemical Properties of Maize (Zea mays L.)

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Abstract: The fermentation of maize serves as a major source of nourishment for large rural population. It contributes significantly to food security by increasing the range of raw material which can be used in the production of edible products. Preliminary biochemical studies on the fermentation of Zea mays L. were carried out using milled grains. The proximate analysis showed that the sample contains the following components; crude protein, lipid and reducing sugar. The experiment showed that these components increased in concentration as the period of fermentation is increased. Vitamin analysis showed that Vitamin C is also contained on the sample and that fermentation enhanced Vitamin C contents. Antinutritional assessment showed that tannin and phytate are present at reduced concentration and they decreased as the period of fermentation is increased. It is concluded that the quality of food could be improved by fermentation.

Key words: Rural population - Vitamin C - Proximate analysis and Food safety

INTRODUCTION

Fermentation is one of the oldest applied biotechnology techniques. It has been used in food preservation for over 6,000 years. It is an inexpensive and manageable food preservation technique and very appropriate where other processing techniques such as canning and freezing are either inaccessible or non-existent [1-3]. Fermentation processing is also labour intensive with minimal infrastructural, energy requirements and is well integrated into village life in rural areas of many developing countries. There is a need to ensure that fermentation is not displaced by economic and cultural changes and that the knowledge base of their production is not lost. Furthermore, a better understanding of fermentation technologies is needed in order to improve the safety, yields and quality of fermented food products, while consumers need to be educated of the benefits inherent in consuming fermented food [4].

Fermentation enhances the nutritional content of foods through biosynthesis of vitamins, essential amino acids and proteins by improving protein and fibre digestibility. It also provides source of calories when used in the conversion of substrates, unsuitable for action processes. It enhances food safety by reducing toxic compound such as aflatoxins, cyanogens and producing antimicrobial factors such as lactic acid, carbondioxide, hydrogen peroxide and ethanol which facilitates inhibition or elimination of food borne pathogens [1]. Therapeutic properties of fermented foods have also been reported. In addition to nutritive, safety and preservation effects, fermentation enriches the diet through production of a diversity of flavours, textures and aromas. It improves the shelf life of food by reducing energy consumption required for their preparation.

Traditional fermentation process is generally a spontaneous, non-aseptic operation which, as a results of the competitive activities of different micro-organisms. In bio-reactors, which may consist of clay or metal pots, baskets, or simply hole in the ground lined with leaves, strains best adapted and with the highest growth rates, dominate under uncontrolled condition. Improvement in process control through the development of more appropriate bioreactors, particularly those suitable for solid substrate fermentation, could improve the quality and quantity of fermented food available in developing countries. The selection and development of more productive microbial strains and the control and manipulation of culture conditions could also increase the efficiency of fermentation processes [5].

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Maize is a cereal that is produced abundantly in Nigeria. In the 1978/79, about 2.4 million tones of cereal were produced. During the same period, maize farming represented about 60% of the total cultivated plant in Nigeria [6]. Maize grains are variously used in making of pap and porridges and in traditional beer brewing in Africa.

The grains are the major cereal consumed in the Northern States of Nigeria. Maize seeds have been reported to have low protein contents [6]. Fermentation has been acknowledged for centuries as a natural means of improving the nutritional quality of grains [7]. Foods have from antiquity been fermented to extend shelf life and in some cases improve its level of safety [8]. Fermented foods are important components of diets in many parts of the world, especially Africa and Asia. Sometimes, the final products make important contributions to the diet as sources of proteins, energy and some vitamins [9].

As a result, of the impact of fermentation in grains used in making some local foods, the aim of this research was to determine the effect of fermentation on some selected biochemical properties of Zea mays L.

MATERIALS AND METHODS

Material: Yellow maize seeds Zea mays L. were used for this analysis. The maize seeds were purchased from a local retailer at Nsukka Market, Enugu State, Nigeria.

Preparation of Plant Materials: The maize grains were washed properly and soaked in a bowl for three days. The water (distilled) was always changed every morning to maintain freshness and also to avoid contamination of the maize.

They were washed very well on the third day and were then ground with fresh distilled water. The ground grains were sieved with distilled water and this was added to enhance the flow of wanted particles through the sieve and hence leaving the chaff on top of the sieve. The filtrate was then divided into two equal parts [A and B] in two bowls and 10ml of distilled water was added to bowl A only while bowl B was used like that for the analysis.

Determination of pH: The determination of pH of the sample was done from the first day of fermentation, i.e. the day the grains were ground, sieved and divided into two equal portions to the last day of the fermentation process, i.e., the sixth day of the fermentation.

The pH of sample A was determined in triplicate each day until the sixth day. The same was done for sample B. In the second day of the fermentation, the water in sample B was changed while that of sample A was not changed and their pH were determined in triplicate. This procedure was the same for each day.

Proximate Analysis: This analytical procedure was used to determine the approximate values of the different components or constituents of the grains. It can also be used to determine other food constituents. This is properly used for the assessment of the various food compositions. This analytical procedure was carried out on the fermenting grains to evaluate the compositions of the grains such as protein, fat and oil and reducing sugar, etc.

Determination of Lipid: The determination of lipid was carried out by the Soxhlet method of lipid extraction. In this method, an extraction cup was weighed and 5 ml of the sample A was added to cup and weighed. This was done in triplicate from the first to the sixth day of fermentation. The same was also done with sample B, i.e. the sample in which water was being changed every morning. These samples in triplicate were then placed into an extraction thimble. The thimble with the cup was placed inside the soxhlet apparatus with a solvent flask containing 250ml hexane. It was heated for 60 min to enhance extraction.

The cups and thimble were then removed and left to cool at room temperature. The final weights of the different cups were obtained and the weights were used to calculate the percentage of the lipid in the sample using the following formula of Ramgopal et al. (2010).

Calculation:

\[
\% \text{ of lipid} = \frac{B - C}{A} \times 100
\]

Where,

B=weight of cup + sample
C=Final weight of cup after extracts
A=Volume of sample used.

By substituting for A, B. and C, we have \% of lipid = weight of cup + sample – final weight of cup after extraction volume of sample used.
**Determination Crude Protein:** 5 ml of sample A was pipetted into a Kjeldahl digestion flask of 100 ml capacity. After that, 1 g of cupric sulphate and 3 g of sodium sulphate were added. Cupric sulphate and sodium acted as a catalyst by speeding up the reaction. This was mixed properly by shaking the 100ml capacity flask carefully.

This preparation was done in triplicate from the first to the sixth day of the fermentation. The same thing was also done for sample B. The flasks, filled with loose-pear-stoppers in an inclined position, were heated in fume cupboard. The flasks were shaken from time to time to ensure that the contents of the flask were properly mixed up. The sample was digested until a clear blue solution was got at approximately an hour. The flasks were brought down and allowed to cool.

The digests were volumetrically transferred to another 100 ml volumetric flask and made up to the mark. The distillation unit was steamed out to prepare the unit for use. 5 ml of the digest was pipetted into the digestion chamber, 5 ml of 6% NaOH was also added into the chamber to give a mixture. The steam was passed through the reaction mixture and distilled for about 30 minutes with the pinch-corks closed. Ten ml of boric acid was mixed with methyl red and methyl blue, indicators to give a boric acid mixed indicator, which was placed in 100 ml receiving flask.

The end to the condenser tube was placed below the surface of the boric acid mixed indicator so that this acid could trap the ammonia gas content in the distilled digestion sample. A colour change was observed (blue to green) on the receiving flask and then the flask itself was covered so that the condenser tube was just above the boric acid mixed indicator and distillation was continued for 5 min to ensure complete distillation of all the ammonia. The distillate from the receiving flask was removed and taken to the table for titration.

This procedure was also repeated for the other two samples of sample A and then for the three samples of sample B from the first day to the sixth day.

The titration was done with 0.01 N HCl and change in colour was observed, i.e. from blue to green

**Calculation:**

Normality of HCl = 0.01

Dilution = 50

% of Nitrogen = \( \frac{Titre \times 0.01 \times 100}{Volume \ of \ sample} \)

% of crude protein = % Nitrogen x 6.25

6.25 is the nitrogen protein factor.

**Determination of the Percentage of Reducing Sugar:** Phenol method of determination of reducing sugar was carried out to estimate the reducing sugar content of the grains. Twenty ml of the sample was pipetted into a beaker and was allowed to boil in a water bath for 10 minutes. After that, it was brought down and allowed to cool. Ten ml was pipetted from the beaker into 100 ml volumetric flask and diluted to the mark with distilled water. This was mixed thoroughly and 2 ml aliquot was pipetted from the flask into a test-tube. One ml of 5% phenol was added into the test-tube and it was mixed gently. Five ml of concentrated sulphuric acid was also pipetted into the test tube.

The test tube with mixtures was allowed to stand for 10 minutes, shook to mix well and was immersed in a water bath at 30°C for 20 minutes. A yellow-orange colour was formed. The absorbance was measured in a spectrophotometer at 500 nm and value was taken. This was done in triplicate for both sample A and B for six days.

**Determination of Vitamin C:** Five ml of the grains was pipetted into 100 ml volumetric flask and 25 ml of 0.05% metaphosphoric acid which acted as a stabilizing agent was also added and the mark was made up with distilled water. Ten ml of the solution was pipetted into another 100 ml volumetric flask and 2 ml of acetone was added. The indophenol solution (0.05.2, 6-dichloroindophenol dissolved in distilled water and diluted in 100 ml and filtered) until a faint pink colour persisted for 15 seconds. The solution of a faint pink colour was then measured spectrophotometrically to determine the Vit. C contents of the sample at 550nm. This was done in triplicate and for both samples A and B for six days.

**Calculation:**

\[
\text{Vit. C} = \frac{AB \times \text{factor} \times \text{vol developed} \times 100}{\text{Vol of same}}
\]

Where, dilution factor = 20

Volume used to dev. Colour = 10

Optical density = 550 nm.

**Assessment of Antinutrients and Toxic Factors:** Determination of Phytic Acid: The quantitative analysis or determination of phytic acid was by the method of Latta and Eskin (1980). Five ml of the sample was pipetted into a 500 ml flat bottom flask. The flask with the sample was placed in a shaker and extracted with 100 ml 2.4% HCl
for 1 hour at room temperature and was then diluted to 10 ml in another test tube with distilled water and colour was developed with one ml wade reagent solution.

The wade reagent solution is a mixture of 0.03% of FeCl₃ and 0.3% sulphosalicylic acid. The colour solution in a test tube developed with wade reagent was read at 500 nm in a spectrophotometer. This was done in triplicate for samples A and B for six days.

Determination of Tannic Acid: The quantitative determination of tannic acid was by method of vanillin HCl of Price and Butter (1980). Five ml of the sample was pipetted into test tube and 10 ml of 2M HCl was added to it and shaken for 5 minutes. The content was quantitatively transferred into 50 ml volumetric flask and made up to 50 ml and filtered. Five ml of the filtrate was pipetted into a test and 3 ml of 0.1 ml FeCl₃ in 0.1 HCl and 3 ml of 0.008 M potassium ferricyanide (K₃Fe(CN)₆) were added to the filtrate. The test tube was read in a spectrophotometer at 650 nm. This was done in triplicate and for the samples and for six days.

RESULTS

pH Determination of Sample A and B: The results of the pH for the sample A and B of the maize grains are as follows:

pH Determination of Sample A and B: The table 1 shows the result of pH for the sample A and B of the maize grains.

Table 1: pH Values of Sample A and B

<table>
<thead>
<tr>
<th>Day</th>
<th>Mean pH Values for sample A</th>
<th>Mean pH Values for sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.70±0.0082</td>
<td>5.70±0.0082</td>
</tr>
<tr>
<td>2</td>
<td>4.97±0.0005</td>
<td>4.8975±0.0096</td>
</tr>
<tr>
<td>3</td>
<td>3.925±0.0096</td>
<td>3.9375±0.0096</td>
</tr>
<tr>
<td>4</td>
<td>3.62±0.005</td>
<td>3.5725±0.0096</td>
</tr>
<tr>
<td>5</td>
<td>3.32±0.0265</td>
<td>3.3925±0.0096</td>
</tr>
<tr>
<td>6</td>
<td>3.01±0.012</td>
<td>2.505±0.4968</td>
</tr>
</tbody>
</table>

Proximate Analysis for Sample A: The results of the proximate analysis for sample A were as follows:

From the table 2 above, there seems to be changes in the values of lipid and protein but for reducing sugar, Vit. C, there values increased as the fermentation was increased.

These values were the % composition (mean).

Proximate Analysis for Sample B: The results of the proximate analysis for sample B were as follows:

From the table 3, there are varied values of lipids and crude protein but reducing sugar and Vit. C. maintained a kind of uniform decrease and increase respectively.

These values are % compositions (mean).

Assessment for Antinutrients and Toxic Factors: The results of the assessment for sample A are as follows:

Table 2: Values of Proximate Analysis for Sample A

<table>
<thead>
<tr>
<th>Components (%)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>0.1475±0.0096</td>
<td>0.27±0.0258</td>
<td>0.19±0.0082</td>
<td>0.21±0.0082</td>
<td>0.35±0.0082</td>
<td>0.41±0.0115</td>
</tr>
<tr>
<td>Crude protein</td>
<td>2.2125±0.1427</td>
<td>6.475±0.2630</td>
<td>6.7975±0.0818</td>
<td>3.70±0.0816</td>
<td>3.95±0.10</td>
<td>4.15±0.0577</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>0.48±0.0082</td>
<td>0.4125±0.0096</td>
<td>0.38±0.005</td>
<td>0.385±0.0058</td>
<td>0.35±0.0082</td>
<td>0.167±0.0189</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.6075±0.0096</td>
<td>2.125±0.0957</td>
<td>2.43±0.0153</td>
<td>2.445±0.0208</td>
<td>2.65±0.0222</td>
<td>2.985±0.0096</td>
</tr>
</tbody>
</table>

Table 3: Values of Proximate Analysis for Sample B

<table>
<thead>
<tr>
<th>Components (%)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid</td>
<td>0.155±0.01</td>
<td>0.2725±0.0222</td>
<td>0.1175±0.005</td>
<td>0.175±0.0058</td>
<td>0.23±0.0082</td>
<td>0.2675±0.0055</td>
</tr>
<tr>
<td>Crude protein</td>
<td>2.025±0.05</td>
<td>6.575±0.095</td>
<td>7.25±0.0577</td>
<td>3.85±0.577</td>
<td>2.7075±0.0096</td>
<td>3.80±0.0082</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>0.3875±0.005</td>
<td>0.375±0.0058</td>
<td>0.305±0.009</td>
<td>0.2925±0.005</td>
<td>0.2525±0.005</td>
<td>0.18±0.0082</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1.605±0.0058</td>
<td>1.80±0.1155</td>
<td>1.987±0.005</td>
<td>2.01±0.0055</td>
<td>2.25±0.005</td>
<td>2.5525±0.0096</td>
</tr>
</tbody>
</table>

Table 4a: Values of Antinutrients and Toxic Factors for Sample A

<table>
<thead>
<tr>
<th>Components (%)</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate (%)</td>
<td>0.235±0.0058</td>
<td>0.1475±0.005</td>
<td>0.1475±0.005</td>
<td>0.212±0.005</td>
<td>0.1925±0.005</td>
<td>0.165±0.0058</td>
</tr>
<tr>
<td>Tannin (%)</td>
<td>0.2425±0.0096</td>
<td>0.21±0.0082</td>
<td>0.185±0.0058</td>
<td>0.15125±0.005</td>
<td>0.1225±0.005</td>
<td>0.12±0.0141</td>
</tr>
</tbody>
</table>
Table 4b: Values of Antinutrients and Toxic Factors for Sample B

<table>
<thead>
<tr>
<th>Components</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate (%)</td>
<td>0.2325±0.005</td>
<td>9.175±0.0058</td>
<td>0.16±0.0082</td>
<td>0.2875±0.005</td>
<td>0.245±0.0058</td>
<td>0.1875±0.005</td>
</tr>
<tr>
<td>Tannin (%)</td>
<td>0.24±0.0082</td>
<td>0.2075±0.0096</td>
<td>0.1875±0.005</td>
<td>0.17±0.0082</td>
<td>0.152±0.0096</td>
<td>0.15±0.0082</td>
</tr>
</tbody>
</table>

These values are % composition (mean).

From the table 4a, the results show that the maize grains contain low phytate and tannin.

**Analysis of Sample B for Antinutrient and Toxic Factors:**

The results from sample B are shown below.

From the table 4b above, the values varied and also phytate and tannin were low in concentration of the maize analysed.

These values are % composition (mean).

**DISCUSSION**

The nutrient compositions of maize grains are dependent on the relative proportion of different components such as fat and oil, protein and Vit. C etc. It varied considerably with genetic background, environmental conditions, processing and analytical procedures [4, 3, 6, 10].

The lipid content of the samples varied and the variation were a function of fermentation periods. There were decreases among the low values of lipid. The low lipid levels or values for the sample suggest the keep ability of the maize seeds. This is because the lower lipid content of a given food substance, the higher keeping of the maize seeds. This is because the lower lipid content of a given food substance, the higher keeping quality and vice versa [11, 12].

The protein content of the sample varied. The variation ranges from 2.02-2.25%, the highest value of protein was on the third day of fermentation. The highest protein content might be when there is optimum condition of fermenting organisms for synthesis of protein from hydrolysed amino acids and peptides. Protein concentration in the fermentation medium started to increase from the first day of fermentation and rather, large increase was observed between the first and second day of fermentation. This rapid increase could correspond to a period of very rapid microbial multiplication [13].

The experiment shows that the Vit. C. content of the sample increased as the fermentation increased. The range of variation is 1.605 to 2.98%, which was found to be at the first and six days of fermentation. It also shows that the concentration of free reducing sugar may be attributed to the microbial utilization. A show rate of enzymatic hydrolysis could be the limiting factor to the production of high reducing sugar concentration [7].

The experiment also shows that tannin and phytate were very well reduced in their concentrations. The range of variation of phytate is 0.16-0.28%, the lowest value was got on the third while the highest value was got on the fourth day of fermentation. Also the range of variation of tannin was 0.15 to 0.24%, the lowest value was got on the third day while the high value was got on the six day.

It is known that tannins which interferes with nutrient availability were drastically reduced by milling [9, 14]. The increase in phytate indicates that milling was not beneficial and that the rural dwellers would be properly advised against the use of milling as the only method of reducing phytate in grains during fermentation, phytate is inactivated. This enzyme hydrolyzes protein-mineral-phytate complexes to release more free protein and subsequently phytate is lost in the fermenting medium and subsequently phytate is the fermenting medium [14]. This loss has been reported by workers to account for the decrease in phytate during optimum fermentation and sieving of maize grain may be due to hydrolysis of phytate-mineral complexes and small particle size due to sieving. The enzyme hydrolyzes phytate to inositol and phosphoric acid. This reduces phytate levels and increased mineral and other nutrients [14].

The results of pH determination show that at the start of the experiment that the pH of the fermenting sample was high but at the end of the experiment, the pH of the fermenting sample was low. This shows that there was high acidification of the culture medium during fermentation. The high acidification may be due to some products of micro-organisms during the periods of fermentation.

**CONCLUSION**

Although some fermentation are undesirable or abnormal because they cause spoilage of foods and beverages. The present study has shown that fermentation increases the nutritive values or qualities of grain such as vitamins which are essential especially for the growing children. Fermentation led to increases in protein especially at the beginning of the process.
Also, analysis shows that the fermentation processes are desirable because it improves the palatability and texture, to extend shelf life and keep ability. This is due to the low content of lipid in the grains.

**Recommendation:** It is necessary that appropriate processing technique for grains be developed for maximum elimination of antinutritional factors and toxic factors that affect the availability of nutrient in foods and grains.

**REFERENCES**