EFFECT OF FERMENTATION ON NUTRITIONAL COMPOSITION OF SELECTED COMMONLY CONSUMED GREEN LEAFY VEGETABLES IN NIGERIA

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Abstract

Fresh leafy vegetables which include Amaranthus hybridus (Bush green), Telfaria occidentalis (Fluted pumpkin leaf), Vernonia amygdalina (Bitter leaf) and Pterocarpus mildbraedii (White campwood leaf) were subjected to spontaneous fermentation for 5 days at room temperature. Physicochemical properties (pH, temperature, and total titratable acidity) were monitored during the fermentation. It was observed that the pH (6.8 to 3.78) of all the vegetables decreased steadily as fermentation progressed. However, the temperature (27°C to 34°C) and total titratable acidity expressed as lactic acid equivalent (0.014-0.147) for all the vegetables increased as fermentation progressed. The percentage of occurrence of bacteria isolated from the fermenting mass include Bacillus spp. (39.1%), Lactobacillus spp. (26.1%), Escherichia coli and Staphylococcus spp. (8.7%), Proteus spp., Serratia spp., Citrobacter spp. and Pseudomonas spp (4.3%) respectively. The proximate composition and mineral contents of fermented and unfermented leaves were investigated. Significant increase in ash content was noticed for unfermented (8.07%-15.72%) and for fermented vegetables (12.50%-23.28%). The protein contents of T. occidentalis (24.29%-25.65%), and A. hybridus (14.27%-16.96%) increased significantly after fermentation. A decrease in fiber content was noticed for all fermented vegetables except for V. amygdalina where significant increase was noticed (10.97%-14.55%). Increase in both thiamine (1.37-1.52mg/ml) and niacin (1.32-1.62mg/ml) contents were observed in T. occidentalis after fermentation while significant reductions were recorded for A. hybridus and V. amygdalina. Generally, vitamin C content of the vegetables decreased during fermentation while some increases were recorded in the mineral content of fermented vegetables.

Key Words: Amaranthus hybridus; fermentation; nutritional composition; Pterocarpus mildbraedii; Telfaria occidentalis; Vernonia amygdalina

Introduction

Vegetables are agricultural products which are important sources of protective foods that are highly beneficial for the maintenance of good health and prevention of diseases (Shade et al., 2004). Although some vegetables can be raised comparatively at lower management costs and on poor marginal soil, they have remained underutilized due to lack of awareness of their nutritional values in favor of exotic vegetables (Odhav, 2007). Leafy vegetables represent an inexpensive but high quality nutritional source especially for the poor segment of the population where malnutrition is wide spread.

Bitter leaf (Vernonia amygdalina) has found relevance in traditional folk medicine as anthelminitics, anti-malarial, antimicrobial anticancer and as a laxative herb (Huffman, 2003). The leaves are used as green leafy vegetable and may be consumed either as a vegetable (leaves are macerated in soups) or aqueous extracts used as tonics for the treatment of various illnesses (Huffman, 2003). Many herbalists and native doctors in Africa recommend its aqueous extracts for their patients as treatment for varieties of ailments ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexually transmitted diseases and diabetes mellitus among others (Argheore et al., 1998), and for fevers and are known as a quinine—substitute (Masaba, 2000).

White campwood (Pterocarpus mildbraedii) leaves are used as vegetables in the preparation of soup in Nigeria, in Ghana; the trees have been extensively utilized in cocoa plantations to provide shade (Bosch, 2004). Some tribes in Eastern and Southern Nigeria use the leaf extracts from Pterocarpus mildbraedii in the treatment of headaches, pains, fever, convulsions, and respiratory disorders and as antimicrobial agents (Ogukwe et al., 2004).

Bush green (Amaranthus hybridus) are spread throughout the world growing under a wide range of climatic conditions and they are able to produce grains and leafy vegetables (Giriya et al., 2011). One of the reasons there has been recent interest in amaranth is because of its useful nutritional qualities. The grain has some protein (12 % to 17 %) and is...
high in lysine, an amino acid that is low in other grain crops. The grain is high in fibre and low in saturated fats, factors which contribute to its use by the health food market (Girija et al., 2011). In Nigeria, *Amaranthus hybridus* leaves combined with condiments are used to prepare soups. In Congo, the leaves are eaten as spinach or green vegetable when boiled and mixed with a groundnut sauce (Dhellot et al., 2006).

Fluted pumpkin leaves (*Telfaira occidentalis*) is rich in protein and minerals (Aleotor et al., 2002), reported to attenuate the testicular damage induced by quinine (Nwangwa et al., 2007) and also found to reduce lipid peroxidation thereby improving spermatogenesis (Emeka and Obidoa, 2009). The aqueous extract of *T. occidentalis* has been shown to be hepatoprotective against garlic-induced oxidative stress (Olorunfemi et al., 2005).

Vegetables present unique problems which negatively confront attempts to extend their postharvest useful life span on account of their fragile texture and high moisture contents, both of which are responsible for their rapid deterioration and drying difficulties with loss of heat sensitive nutrients. The nutritional and biological potential of vegetable juices due to their mineral and vitamin content and good results in prophylaxis has been documented (Moraru et al., 2007). The probiotic effect of fermented vegetable juices could be an option to lactose intolerance and cholesterol content of yoghurt and other dairy products (Luckow and Delahunty, 2004). Several researches have shown that fermentation enhances the nutrient content of foods through the biosynthesis of vitamins, essential amino acids, micronutrient bioavailability and aids in degrading antinutritional factors (Oboh and Akindahunsi, 2003; Oboh 2006). The objective of this study is therefore to investigate the microorganisms involved in the natural fermentation of *Amaranthus hybridus*, *Vernonia amygdalina*, *Telfaira occidentalis* and *Pterocarpus mildbreadii* and the effect of fermentation on the nutritional properties of the vegetables.

**Materials and methods**

**Sample preparation**

Fresh green leafy vegetables which include *Amaranthus hybridus* (Bush green), *Telfaira occidentalis* (Fluted pumpkin leaf), *Vernonia amygdalina* (Bitter leaf) and *Pterocarpus mildbreadii* (White campwood leaf) were purchased in a local market in Akure, Ondo State Nigeria. Authentication of the leaves were carried out in the Department of Crop Science and Pest Management, Federal University of Technology Akure, Nigeria

The leaves were destalked and sorted, washed in water, drained and chopped into small pieces. Certain portions were dried without fermentation. Salt was added to the other portions (2g of salt to 100g of vegetables) and mixed thoroughly before packing into plastic buckets which were covered tightly to serve as fermenting jars. Fermentation was allowed to proceed at room temperature for five days and the process was monitored by measuring pH using a pH meter, temperature and total titratable acidity (TTA). After fermentation, the samples were separately oven dried (50°C), blended with a laboratory blender, and stored in labeled air tight plastic containers for further analyses.

### Isolation of microorganisms from the fermented vegetables

Twenty five grams of each vegetable sample was homogenized with 225ml of buffered peptone water (Difco Labs, Division of Becton Dickinson and Co., Sparks, Md., U.S.A.). Serial dilutions were performed and from the appropriate dilutions, 1ml each was drawn and pour plated using de Mann Rogosa and Sharpe (MRS), Nutrient agar, and Potato dextrose agar respectively. For total viable count, plates were incubated for 24hours at 30°C. For the LAB, the plates were incubated in an anaerobic jar for 48 hours. Yeasts and molds on the PDA plates were incubated at room temperature for 72 hours. Pure isolates were subjected to further tests for characterization following Bergey’s manual of Determinative Bacteriology (Holt et al., 1994).

### Determination of Proximate composition of fermented and unfermented (raw dried) vegetables

The proximate composition of the vegetables (moisture, ash, fiber, protein and carbohydrate) was determined using the method of AOAC (2005).

### Determination of Niacin (vitamin B3) content of fermented and raw dried vegetables

Five grams of the sample was treated with 50ml of 1N H2SO4 and shaken for 30min. Three drops of ammonia solution were added to the sample and filtered. Ten milliliter of the filtrate was pipetted into a 50ml volumetric flask and 5ml of potassium cyanide was added. This was acidified with 5ml of 0.02N and absorbance was measured using spectrophotometer at 470nm (Okwu and Josiah, 2006).

### Determination of Thiamine content (vitamin B1) of fermented and raw dried vegetables

Five grams of the sample was homogenized with 50ml ethanolic sodium hydroxide. It was filtered into a 100ml conical flask 10ml of the filtrate was pipette and the color was developed by addition of 10ml of potassium dichromate and read at 360nm. A blank solution was also prepared following the method of Okwu and Josiah (2006).

### Determination of vitamin C content of fermented and raw dried vegetables

The vitamin C content of the hydrophilic extract was determined using the method of Benderitter et al.(1998). About 75µl DNPH (2g dinitrophenyl hydrazine, 230mg thiourea and 270mgCuSO4.5H2O in 100ml of 5M H2SO4) was added to 500µl reaction mixture (300µl appropriate dilution of hydrophilic extract with 100µl of 13.3% trichloroacetic acid and water). The reaction mixture was...
subsequently incubated for 3hr at 37°C, then 0.5ml of 65% H_2SO_4 (v/v) was added to the medium, the absorbance was measured at 520nm, and the vitamin C content of the sample was subsequently calculated.

Determination of mineral content of fermented and raw dried vegetables
The mineral content of the samples were determined using the Energy dispersive X-ray fluorescence spectroscopy (EDXRF). The sample was placed on a rotating tray; a high power X-ray tube irradiates a metal disc causing it to emit its own characteristic radiation lines. This fluorescent radiation excites the samples in the tray and energies are emitted. The spectra are shown on a detector which is capable of separating and measuring the different energies of the characteristic radiation emitted from the sample to determine the elements present. How much of a particular element present is determined by measuring the intensity of the emitted energies (Jerkins, 2000).

Statistical analysis
All Analyses were carried out in triplicates. The results obtained were subjected to analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) version 17.0. Means were separated using the Duncan multiple range test (DMRT) at 95% confidence level (p<0.05).

Results and Discussion
The total titratable acidity of vegetables during fermentation is presented in Fig. 1. The titratable acidity increased as fermentation progressed. The total titratable acidity ranged between 0.035-0.133 for Telfairia occidentalis, 0.014-0.098 for Vernonia amygdalina, 0.042-0.112 for Amaranthus hybridus and 0.035-0.147 for Pterocarpus mildbreadii. The increase in total titratable acidity observed is in accordance with the findings of Oyewole and Ogundele (2001) that attributed the increase to the production of organic acids during fermentation. There were increases in the temperature as fermentation progresses (data not shown). The temperature of all the vegetables increased as fermentation progressed and ranged from 27 °C to 34 °C. Fig. 2 showed the pH profile of vegetables (6.8 to 3.78) during fermentation and the pH of all the vegetables decreased steadily as fermentation progressed. The pH values obtained in this study were higher as compared to sauerkraut with a final pH of 3.4-4.0. However, the steady pH reduction tallies with that reported for fermented Amaranthus hybridus (Mariga et al., 2011). The sudden pH drop may provide pH shock and prevent the survival of both pathogenic and spoilage organisms in the vegetable.

Table 1 revealed the bacterial succession during fermentation of the vegetables for 120 hours which include Bacillus spp., Lactic acid bacteria, Escherichia coli, Staphylococcus spp., Proteus spp., Serratia spp., Citrobacter spp. and Pseudomonas spp. In addition, fungi isolated from the vegetables (Penicillium spp., Fusarium spp., Aspergillus spp., Neurospora spp., Geotrichum spp., Rhizopus spp.) and Saccharomyces spp are shown on Table 2. Bacillus spp. remained a dominant flora throughout the fermentation followed by lactic acid bacteria. Bacillus spp. have been reported to be responsible for the fermentation of African locust bean and other oil seeds to produce condiment (Achi, 2005). Escherichia coli was found on the day zero of the fermentation but was not present again as the fermentation continued. Some of the lactic acid bacteria isolated have been reported as probiotics thus suggesting that fermented vegetables can serve as a functional food by exhibiting other health beneficial functions such as stabilizing the gastrointestinal tract (Gorbach,2002)

Fig. 1: Total titratable acidity of vegetables during fermentation
Legend:
T.O = Telfairia occidentalis; V.A = Vernonia amygdalina
A.H = Amaranthus hybridus; P.M = Pterocarpus mildbreadii

Fig. 2: pH profile of vegetables during fermentation

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The result of the proximate analysis of the raw dried and fermented vegetables is presented on Table 3. The ash content of all the vegetables increased after fermentation. The ash content ranged from 8.07%-15.72% for freshly dried vegetable to 12.50%-23.28% for fermented samples while the protein content ranged from 14.55%-30.67% for freshly dried samples to 16.96%-25.89% for fermented samples. This compared favorably with the percentage of dry milled values reported for *Telfairia occidentalis* (22.4%), *Tonamndus indica* (24.3%) and *Parkia biglobosa* (20.9%) (Igba et al., 2006). The results obtained for both fermented and unfermented vegetables can also be compared to protein rich foods such as soybeans, cowpea, melon and pumpkin (23.10-33.00%) as reported by Omoyeni and Adeyeye (2009. The fat contents of *V. amygdalina* and *P. mildbreadii* were not affected by fermentation. There was however a significant increase in the fat content of *T. occidentalis* (8.33%-10.05%) and *Amaranthus hybridus* (4.74%-8.80%). The changes in nutrient composition during fermentation of oil seed have been facilitated by enzymatic activities of fermenting organisms (Enujuigha, 2003). The fat contents of *A. hybridus*, *P. mildbreadii* and *V. amygdalina* which was between 4.74% to 8.80% are within the percentage values reported for leafy vegetables such as *Brachystegia eorycoma* (5.78%) and *T. indica* (4.2%) (Ajayi et al., 2006). The fibre content of the vegetables ranged from 10.03%-14.55% and this exceeded the fiber content reported for *Talium triangulare* (2.0%) and *T. occidentalis* (1.7%) (Akachukwu and Fawusi, 1995). This may be an indication...
that the roughage content of these plants is high and will promote digestion and prevent constipation. Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol (Mensah et al., 2008). After fermentation there were reductions in the carbohydrate content (29.06%–50.02%) of the four vegetables. The result revealed that the carbohydrate content of the fermented vegetables were in the range reported for Ocimum basilicum (36.41%) and O. grattissimum (35.61%) (Iifesan et al., 2006). However, both the values for fermented and unfermented vegetables were higher than that of Aerva lanata (26.6 g) (Omonyeni and Adeeye, 2009). The high carbohydrate content of vegetables may suggest that they can be good sources of energy and also assist in efficient oxidation of fats.

Table 4 showed the Vitamin C, thiamine and niacin contents of the unfermented and fermented vegetables. Generally, there were reductions in the vitamin C contents of the vegetables except for A. hybridus (0.08 mg/ml-0.13 mg/ml) after fermentation. This may be due to the processing methods used in this study. However, Vitamin C has been shown to be better preserved in fermented vegetable products when compared with other alternative methods (Nout and Motarjemi, 1997). Ascorbic acid contributes to the antioxidant properties of vegetables by protecting the membrane erythrocyte, maintaining the blood vessel flexibility and improving blood circulation in the arteries (Oboh, 2005). It was observed that there were increases in both thiamine (1.37 mg/ml-1.52 mg/ml) and niacin (1.32 mg/ml-1.62 mg/ml) contents of T. occidentalis after fermentation while reductions were obtained for all other vegetables investigated. Niacin (Vit B3) is a water-soluble vitamin and this may explain why there were reductions in the niacin content of the vegetables during fermentation. Niacin assists the body to use fat, protein and carbohydrates from foods to make energy while thiamine plays a key role in carrying out the metabolic activities of the body system.

The mineral compositions of the raw dried and fermented vegetables showed that the vegetables could be rich sources of most essential minerals (Table 5). It was observed that calcium content were higher in fermented T. occidentalis (7.74 mg/100g-9.11 mg/100g) and V. amygdalina (9.87 mg/100g -11.13 mg/100g). The calcium content of vegetables in this study were higher than those reported for Grylolotalpa africana (4.13 mg/100 g), T. triangulare (7.44 mg/100 g), A. cruentus (2.05 mg/100 g) and Celosia spp. (2.66 mg/100 g) (Mensah et al., 2008). Similar increasing trends were noticed for zinc, magnesium, sodium, potassium, iron, selenium and copper for A. hybridus, V. amygdalina and P. mildbreadii while T. occidentalis showed a slight decrease in value after fermentation. Minerals are also co-enzymes in certain biochemical reactions in the body which underscores the importance of leafy vegetables in metabolic reactions (Mensah et al., 2008).

Table 4: Vitamin C, Thiamine and Niacin (mg/ml) contents of raw dried and fermented vegetables

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Telfairia occidentalis</th>
<th>Amaranthus hybridus</th>
<th>Vernonia amygdalina</th>
<th>Pterocarpus mildbreadii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw dried</td>
<td>0.24±0.005a</td>
<td>0.30±0.04a</td>
<td>0.10±0.005a</td>
<td>0.08±0.001b</td>
</tr>
<tr>
<td>Fermented</td>
<td>0.03±0.001a</td>
<td>0.19±0.05b</td>
<td>0.09±0.005a</td>
<td>0.13±0.001a</td>
</tr>
<tr>
<td>Thiamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw dried</td>
<td>1.37±0.04b</td>
<td>0.76±0.02a</td>
<td>0.67±0.01a</td>
<td>1.26±0.01a</td>
</tr>
<tr>
<td>Fermented</td>
<td>1.52±0.01a</td>
<td>0.17±0.06b</td>
<td>0.45±0.04b</td>
<td>1.22±0.04e</td>
</tr>
<tr>
<td>Niacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw dried</td>
<td>1.32±0.07b</td>
<td>0.78±0.01a</td>
<td>0.47±0.03a</td>
<td>0.27±0.05b</td>
</tr>
<tr>
<td>Fermented</td>
<td>1.62±0.01a</td>
<td>0.22±0.01b</td>
<td>1.26±0.09a</td>
<td>0.95±0.03a</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of three determinations. Differences with different superscripts along columns are significantly different p<0.05

Table 5: Mineral composition (mg/100g) of raw dried and fermented vegetables

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>Zn</th>
<th>Fe</th>
<th>Mn</th>
<th>Se</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>T.O</td>
<td>7.74±2.8</td>
<td>1.81±0.01</td>
<td>6.12±0.005</td>
<td>11.71±0.01</td>
<td>4.89±0.01</td>
<td>7.68±0.01</td>
<td>1.55±0.01</td>
<td>0.55±0.01</td>
<td>2.13±0.05</td>
</tr>
<tr>
<td>T.O(F)</td>
<td>9.11±0.01</td>
<td>1.83±0.01</td>
<td>5.84±0.005</td>
<td>11.0±0.01</td>
<td>4.61±0.01</td>
<td>6.50±0.01</td>
<td>1.32±0.05</td>
<td>0.51±0.005</td>
<td>1.90±0.1</td>
</tr>
<tr>
<td>A.H</td>
<td>10.4±0.05</td>
<td>1.61±0.01</td>
<td>7.01±0.01</td>
<td>12.3±0.1</td>
<td>5.32±0.01</td>
<td>8.12±0.1</td>
<td>1.33±0.01</td>
<td>0.45±0.01</td>
<td>2.96±0.15</td>
</tr>
<tr>
<td>A.H(F)</td>
<td>9.93±0.05</td>
<td>2.01±0.05</td>
<td>8.16±0.1</td>
<td>13.08±0.07</td>
<td>5.97±0.06</td>
<td>9.58±0.02</td>
<td>1.11±0.01</td>
<td>0.51±0.005</td>
<td>3.83±0.11</td>
</tr>
<tr>
<td>V.A</td>
<td>9.87±0.06</td>
<td>1.89±0.005</td>
<td>7.32±0.02</td>
<td>11.54±0.01</td>
<td>4.74±0.03</td>
<td>6.42±0.01</td>
<td>1.30±0.01</td>
<td>0.33±0.01</td>
<td>1.90±0.11</td>
</tr>
<tr>
<td>V.A(F)</td>
<td>11.13±0.57</td>
<td>2.04±0.01</td>
<td>8.95±0.15</td>
<td>12.3±0.06</td>
<td>5.09±0.02</td>
<td>7.79±0.01</td>
<td>1.42±0.04</td>
<td>0.43±0.07</td>
<td>2.00±0.2</td>
</tr>
<tr>
<td>P.M</td>
<td>9.98±0.01</td>
<td>1.88±0.01</td>
<td>6.80±0.01</td>
<td>10.61±0.01</td>
<td>4.4±0.01</td>
<td>6.84±0.04</td>
<td>1.11±0.04</td>
<td>0.38±0.005</td>
<td>1.88±0.1</td>
</tr>
<tr>
<td>P.M(F)</td>
<td>10.6±0.1</td>
<td>2.52±0.01</td>
<td>7.97±0.12</td>
<td>12.53±0.4</td>
<td>5.03±0.07</td>
<td>8.96±0.06</td>
<td>1.11±0.02</td>
<td>0.39±0.01</td>
<td>2.46±0.01</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three determinations and values with different superscripts along columns are significantly different.

T.O= Telfairia occidentalis, A.H= Amaranthus hybridus, V.A= Vernonia amygdalina P.M= Pterocarpus mildbreadii
F = Fermented vegetable

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Conclusion
This study revealed some nutritional value of fermented and unfermented vegetables. It was observed that all the leafy vegetables assayed contained significant amount of mineral content which appeared stable after fermentation. However, there were losses in vitamins. It can be concluded that apart from cabbage, olives and cucumber, some Nigerian leafy vegetables can also be fermented and fermentation can be a preferred processing method to preserve the nutritional component of leafy vegetables.

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