Medbiotech Journal

MBTJ, 2(2): 162-167, 2018

Research Paper

How Nutritional Composition of Commonly Consumed Vegetable Changes under the Influence of Fermentation

Agucha Koko, Lary Yako[1]

Department of Agriculture, University of Ondo State, Nigeria

Received: 19 March 2018 Accepted: 08 May 2018 Published: 01 June 2018

Abstract

In this research, spontaneous fermentation of some selected types of green leafy vegetables including *Telfairia occidentalis* (Fluted pumpkin leaf), *Amarathus hybridus* (Bush green), *Pterocarpus mildbraedii* (White campwood leaf) and *Vernonia amygdalina* (Bitter leaf) was performed for five days at ambient temperature. Changes in physicochemical properties of samples during fermentation were as below: A steady increase was observed in titratable acidity (from 0.014 to 0.147) and temperature (from 27ºC to 34ºC) of all samples, while the pH value was decreased from 6.8 to 3.78. The abundance of isolated bacteria from the fermentation medium was 39.1% *Bacillus spp*., 26.1% *Lactobacillus spp*., 8.7% *Staphylococcus spp*. and *Escherichia coli*, 4.3% *Pseudomonas spp*, *Serratia spp*. and *Citrobacter spp*. Fermentation caused the mineral content of vegetables to increase and the vitamin c content to decrease. Niacin and thiamine content in *A. hybridus* and *V. amygdalina* was decreased, while a reduction was observed for *T. occidentalis. The fiber content of V. amygdalina* showed an increase from 10.97% to 14.55%, while this factor was decreased in other samples. It was also observed that the ash content of fermented and unfermented vegetables was increased from 12.50% to 23.28% and from 8.07% to 15.72%, respectively. The protein content of *A. hybridus and T. occidentalis* increased after fermentation (25.65%-24.29%).

Keywords: Fermentation; Nutritional Composition; Telfairia Occidentalis; Amarathus Hybridus; Pterocarpus Mildbraedii; Vernonia Amygdalina

How to cite the article:

A. Koko, L. Yako, How Nutritional Composition of Commonly Consumed Vegetable Changes under the Influence of Fermentation, Medbiotech J. 2018; 2(2): 162-167, DOI: 10.22034/mbt.2018.76923

1. Introduction

Vegetables are one of the main source of human nutritious foods which can maintain healthy state (Shade *et al*., 2004). Among them, leafy vegetables are of high nutritional value and low cost and so, an appropriate choice for undernourishment poor people. Exotic vegetables, a group of vegetables that are grown in poor soil with low costs, are mainly underused despite their high beneficial health effects (Odhav, 2007).

Beneficial health effect have been proved for fluted pumpkin leaves (*Telfairia occidentalis*). This leafy vegetable that contains high amounts of mineral and proteins [1], can partly compensate quinine-induced testicular damages [2], hepatoprotect against garlic induced oxidative stress (Olorunfemi *et al*., 2005) and improve spermatogenesis by lessening peroxidation of lipids [3].

The other leafy vegetable is bush green (*Amaranthus hybridus*). In Nigeria, people usually mix it with different spices to make soup. It can also be boiled and eaten with groundnut sous, such as what people do in Congo [4]. The grain and green leaves of this plant can arise in various climate all over the world [5]. The high lysine content of amaranth grains, which cannot be found abundantly in other grains, low saturated fat and high amount of fibers make it unique among other grains [5].

[1] Corresponding Author: l.yako.okoko@yahoo.com

Ghanaian people macerate White campwood (*Pterocarpus mildbreadii)* in soups or use it to provide shade on cocoa plant during its growing [6]. *Pterocarpus mildbreadii* is known among tribes in Nigeria because of its antimicrobial effect and also its properties in healing pain, fever, headache, respiratory problems and convulsions (Ogukwe *et al*., 2004).

Bitter leaf (*Vernonia amygdalina*) had a special place among native African doctors and was usually prescribed to cure loss of appetite, nausea, emesis, diabetes, gastrointestinal and sexual transmitted diseases, and also as a treatment for fever instead of quinine [7-8]. This green leafy vegetable can be used in soups or as an aqueous extract with anticancer, anti-malarial, antibacterial, anti-helminthic and laxative properties [9].

Despite the nutritional and beneficial properties of vegetables, their postharvest shelf-life is limited. Drying process of them is along with losing heat-sensitive compounds and also difficult because of high moisture content and fragile texture of the leaves. Vegetable juices possess high amount of vitamins and minerals and so considerable biological and nutritional values [10]. These beneficial properties can be enhanced by fermentation through degradation of anti-nutrition compounds and biosynthesis of amino acids, vitamins and micronutrients (Oboh and Akindahunsi, 2003 and Oboh 2006). In the present work, the physicochemical changes during natural fermentation of *Telfairia occidentalis*, *Amaranthus hybridus*, *Pterocarpus mildbreadii* and *Vernonia amygdalina* were investigated and the involved microorganisms (M.Os) were detected.

2. Materials and Methods

2.1 Materials

Fresh vegetables including *Telfairia occidentalis*, *Amaranthus hybridus*, *Pterocarpus mildbreadii*, and *Vernonia amygdalina* were bought from local markets in Akure, Ondo, Nigeria. These green leafy vegetables were authenticated in Department of Crop Science and Pest Management, Federal University of Technology Akure, Nigeria.

2.2 Methods

2.2.1 Fermentation process

After washing with water, draining and chopping, vegetables were divided in two portions. The first was added 2% salt, mixed and then packed tightly in plastic bags. They were kept at 25 ºC for five days to be fermented. Changes in the pH value, total titratable acidity (TTA) and temperature were detected during fermentation. After that, the fermented vegetables were dried separately in an oven at 50ºC, mixed in a blender, and kept in air-tight plastic container. The second portion of chopped vegetable leaves were dried without fermentation.

2.2.2 M.Os isolation from the fermented medium

To isolate M.Os from the fermented vegetables, 25g of each sample was well mixed with 225ml of buffered peptone water (Difco Labs, Division of Becton Dickinson and Co., Sparks, Md., U.S.A) and then, serial dilution was performed. For microbial cultivation, 1ml of each sample with suitable dilution was pour plated. Potato dextrose agar (PDA) was used to cultivate molds and yeasts at 25ºC for 72h. For total viable count, Nutrient agar plate was used and incubated at 30ºC for 24h. De Mann Rogosa and Sharpe (MRS) plate was used at anaerobic condition for lactic acid bacteria (LAB) growth for 24h.

2.2.3 Determination of Chemical composition

The moisture, protein, carbohydrate, ash and fiber contents of fermented and raw dried vegetables were determined based on the standard method of AOAC (2005).

2.2.4 Vitamins and minerals measurements

To measure the vitamin content in fermented and unfermented (raw dried) samples, spectrophotometric methods were used. Determination of thiamine (vitamin B_1) was done by well mixing of 5g of samples with ethanolic sodium hydroxide and then filtering. Using a pipette, 10ml of sample was poured in a flask and 10ml of potassium dichromate was added to develop the color. Absorbance of samples was then measured at 360nm. The blank solution was prepared as described by Okwu and Josiah (2005).

To measure the niacin (vitamin B_3) content in samples, 50ml of H_2SO_4 (1N) was added to 5g of sample and shook. Then ammonia solution (about 3 droplets) was added to it and filtered. In a volumetric flask, 10ml of the filtrate was poured and 5ml of potassium cyanide was added to it. Acidification was performed using Nitric Acid (0.02N) and then absorbance was read at 470nm (Okwu and Josiah, 2005).

To determine the vitamin C content in the hydrophilic extract, at first, reaction mixture was prepared by addition of 100µl of trichloroacetic acid (13.3%) and water to 300µl of extract. Then 270mg $CuSO_4.5H_2O$, 2g dinitrophenyl hydrazine and 230mg thiourea were added to 100ml H_2SO_4 (5M) to prepare DNPH. In the next step, 500µl of reaction solution was mixed with 75µl DNPH, the mixture was incubated at 37ºC for 3h, and then 0.5ml H_2SO_4 (65% v/v) was added to it. Finally, absorbance of samples was read at 250nm and the vitamin C content was calculated [12].

An Energy Dispersive X-ray fluorescence spectroscopy (EDXRF) was used to determine the mineral content. Sample was put in a tray and radiated by powerful X-ray. Receiving the energy, the sample was excited and emitted radiation. The spectra was analyzed by a detector to determine the type and amount of each element presents in the sample [13].

2.3 Statistical analysis

All experiments were performed in triplicates. SPSS program version 17.0 was used for statistical analysis. Analysis of variance (ANOVA) was performed to determine significant differences between the means. Duncan multiple range test (P <0.05) was used to compare among the means.

3. Results and Discussion

During the fermentation process, the temperature raised from ambient temperature to 34 ºC. TTA (Figure 1) showed an increase trend during fermentation. The range of TTA was 0.035-0.133, 0.014-0.098, 0.042-0.112, and 0.035-0.147 for *Telfairia occidentalis*, *Vernonia amygdalina*, *Amarathus hybridus*, and *Pterocarpus mildbraedii*, respectively. This increase in acidity is due to the organic acids production by M.Os. Same result was reported by Oyewole and Ogundele (2001). Based on Figure 2, the pH value of samples decreased from 6.80 to 3.75 because of the production of organic acids. This sudden decrease can prevent the spoilage M.Os and pathogens. Mariga et al. (2011) reported a similar steady trend of pH decrease in fermentation process of *Amarathus hybridus*. The final pH in our work was higher than that in sauerkraut fermentation (3.4-4.0).

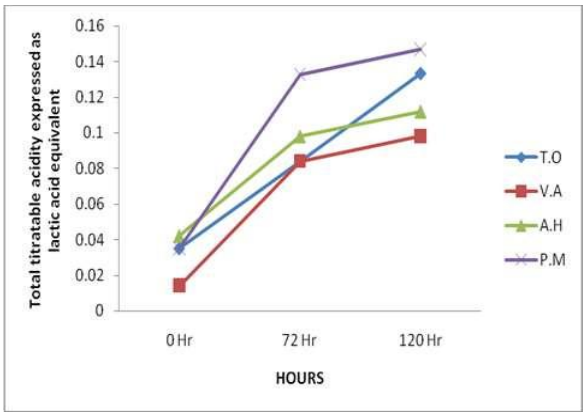

Figure 1. Total titratable acidity of vegetables during fermentation
T.O = *Telfairia occidentalis*; V.A = *Vernonia amygdalina*
A.H = *Amaranthus hybridus*; P.M = *Pterocarpus mildbreadii*

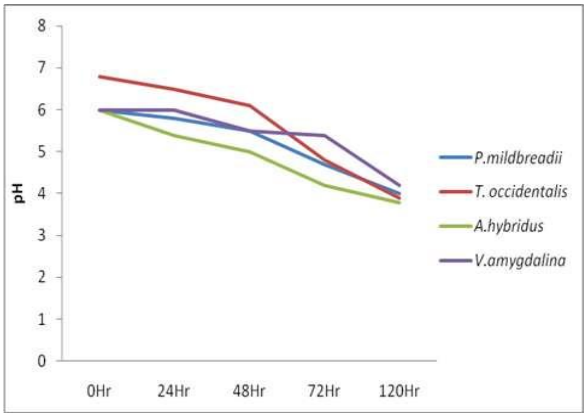

Figure 2. pH profile of vegetables during fermentation

The isolated bacteria involved in the fermentation process and their succession are shown in Table 1. *Bacillus spp.* was the main group of M.Os in fermentation followed by LAB. It has been reported that the responsible M.O for grain seeds fermentation such as African locust bean is *Bacillus spp.* [15]. Considering that some of the isolated LAB are probiotics, the fermentation process can result in a functional product with beneficial effects such as ordering the gastrointestinal tract (GI). On the first day and no longer, *Escherichia coli* was detected in the fermentation medium. The other isolated bacteria during 120h of fermentation include *Serratia spp.*, *Staphylococcus spp.*, *Proteus spp.*, *Pseudomonas spp.*, and *Citrobacter spp*. Table 2 shows that *Saccharomyces spp*. was the only isolated yeast from the fermentation medium and the isolated molds were *Penicillium spp.*, *Fusarium spp.*, *Aspergillus spp.*, *Neurospora spp.*, *Geotrichum spp.*, and *Rhizopus spp*.

Table 1. Bacterial succession during green leafy vegetables fermentation in 120h

Vegetables	Time of isolation (hour)	Probable organisms identified
Telfairia occidentalis	0	*Escherichia coli*, *Citrobacter* spp.
	72	*Bacillus* spp.
	120	*Bacillus* spp. , *Lactobacillus* spp.
Vernonia amygdalina	0	*Staphylococus aureus*, *Serratia* spp.
	72	*Bacillus* spp., *Proteus* spp.
	120	*Bacillus* spp., *Lactobacillus* spp.
Amaranthus hybridus	0	*Escherichia coli*
	72	*Bacillus spp.*
	120	*Bacillus* spp., *Lactobacillus spp.*
Pterocarpus mildbreadii	0	*Pseudomonas* spp., *Staphylococcus aureus*
	72	*Bacillus* spp.
	120	*Bacillus* spp., *Lactobacillus* spp.

Table 2. Fungi isolated from the fermented vegetables.

Vegetables	Moulds Identified	Yeasts Identified
T. occidentalis	*Penicillium spp., Fusarium spp.*	*Saccharomyces spp.*
P. mildbreadii	*Aspergillus spp., Neurospora spp., Fusarium spp., Aspergillus spp.*	*Saccharomyces spp.*
A.hybridus	*Penicillium spp., Geotricum spp.*	*Saccharomyces spp.*
V.amygdalina	*Rhizopus spp., Aspergillus spp.*	*Saccharomyces spp.*

Chemical composition of fermented and unfermented (freshly dried) vegetables are shown in Table 3. As can be seen, fermentation caused the ash content of samples to increase. For unfermented and fermented samples, ash content was in the range of 12.50-23.28% and 8.07-15.72%, respectively. The minimum and maximum values of protein percentage were 16.96 and 25.89 for fermented vegetables and 14.27 and 30.26 for unfermented one, respectively. These amounts of protein are comparable with rich protein food like pumpkin, soybean, melon and cowpea that contain 23.10-33.00% protein (Omoyeni and Adeyeye 2009). Igba *et al*. (2006) reported the protein content of *Parkia biglobosa*, *Telfairia occidentalis*, and *Tonammdus indica* to be 20.9%, 22.4% and 24.3%, respectively. Fermentation process increased the fat content of *Amarathus hybridus* (4.74-8.80%) and *Telfairia occidentalis* (8.33-10.05%), but didn't affect fat content of *Vernonia amygdalina* and *Pterocarpus mildbraedii*. The enzymes of M.Os involved in the fermentation can influence the nutritional value of the products such as oil seeds [17]. In a research, the fat content of *T. indica* and *Brachystegia eorycoma* was determined 4.2% and 5.78%, respectively [18]. The value of fat content for *Amarathus hybridus*, *Vernonia amygdalina*, and *Pterocarpus mildbraedii* (in the range of 4.74%- 8.33%) was close to the report of Ajayi *et al.* (2006). Fibers are beneficial for the body because of reducing the risk of GI cancer and also hinder the over-absorption of cholesterol [19]. The fiber content of vegetables was found to be in the range of 10.03%-14.55%, which is much higher than that of *T. occidentalis* (1.7%) and *Talium triangulare* (2.0%) [20]. So these green leafy vegetable can be consumed for better digestion and prevention of constipation [19]. In addition of being a source of energy for our body, carbohydrates can facilitate the lipid oxidation. It was observed that fermentation caused the carbohydrate content of all samples to decrease because of carbohydrate usage by fermenting M.Os. The value of carbohydrate after fermentation (29.06%-50.02%) was in the range of carbohydrate content for *O. gratissimum* (35.61%) and *Ocimum basilicum* (36.41%) [21]; but higher than the that in the report of *Aerva lanata* (26.6%).

The content of vitamin B_1, vitamin B_3, and vitamin C for freshly dried and fermented green leafy vegetables are presented in Table 3. The metabolism of the body is maintained by the help of vitamin B_1. Energy supplement of the body from carbohydrate, fat, and protein is carried out in the presence of vitamin B_3. *Telfairia occidentalis* showed an increase in vitamin B_1 content (1.37 mg/ml-1.52 mg/ml) and vitamin B_3 content (1.32 mg/ml-1.62 mg/ml) after fermentation, while others showed a decrease. High water solubility of vitamin B_3 can be the reason for its reduction during fermentation process. Vitamin C is an antioxidant that help in blood circulation and keeping the vessels flexible and also protection of membrane erythrocyte. *Amarathus hybridus* showed an increase in its vitamin C content (0.08 mg/ml-0.13 mg/ml), but others showed a reverse trend. Reduction of vitamin C content in this research is probably the result of processing method, but in comparison to the method described by Nout and Motarjemi (1997), this method resulted in higher final content of vitamin C.

Table 3: Proximate compositions (%) of raw dried and fermented vegetables

Parameters (%)		*T.occidentalis*	*V.amygdalina*	*P.mildbreadii*	*A.hybridus*
Ash	Fresh	12.14±1.00^b	12.95±0.38^b	8.070±0.60^b	15.72±0.13^b
	Fermented	14.14±0.10^a	23.28±0.11^a	12.50±0.30^a	21.29±0.06^a
Moisture	Fresh	4.98±0.30^b	3.27±0.02^b	6.33±0.33^a	4.96±0.13^b
	Fermented	6.59±0.48^a	5.80±0.08^a	3.49±0.13^b	5.57±0.25^a
Fat	Fresh	8.33±0.48^b	6.37±0.40^a	6.91±0.26^a	4.74±0.01^b
	Fermented	10.05±0.03^a	7.390±0.13^a	5.580±0.29^a	8.800±0.01^a
Protein	Fresh	24.29±0.01^a	23.51±0.02^a	30.26±0.10^a	14.27±0.04^b
	Fermented	25.65±0.01^a	21.92±0.09^a	25.89±0.17^b	16.96±0.08^a
Fiber	Fresh	12.67±0.22^b	10.97±0.22^b	11.65±0.15^a	11.03±0.15^a
	Fermented	12.90±0.39^a	14.55±0.15^a	10.80±0.10^b	10.03±0.30^b
Carbohydrate	Fresh	36.50±0.09^a	43.12±0.20^a	30.76±0.05^b	50.02±1.20^a
	Fermented	30.67±0.67^b	29.06±0.01^b	41.77±0.03^b	37.35±0.35^b

Values with different alphabets along the rows are statistically significant $p<0.05$

Table 4. Vitamin C, thiamine, and niacin content (mg/ml) of raw dried and fermented vegetables.

Treatments	*Telfairia occidentalis*	*Amaranthus hybridus*	*Vernonia amygdalina*	*Pterocarpus mildbreadii*
Vitamin C Raw dried	0.24±0.005^{a}	0.30±0.04^{a}	0.10±0.005^{a}	0.08±0.001^{b}
Fermented	0.03±0.001^{a}	0.19±0.05^{b}	0.09±0.005^{a}	0.13±0.001^{a}
Thiamine Raw dried	1.37±0.04^{b}	0.76±0.02^{a}	0.67±0.01^{a}	1.26±0.01^{a}
Fermented	1.52±0.01^{a}	0.17±0.06^{b}	0.45±0.04^{b}	1.22±0.04^{a}
Niacin Raw dried	1.32±0.07^{b}	0.78±0.01^{a}	0.47±0.03^{a}	0.27±0.05^{b}
Fermented	1.62±0.01^{a}	0.22±0.01^{b}	1.26±0.09^{a}	0.95±0.03^{a}

Values are mean ± standard deviation after. Different superscripts in each column show significant differences (P< 0.05).

Table 5. Mineral elements (mg/100g) of raw dried and fermented vegetables

	Ca	Mg	Na	K	Zn	Fe	Mn	Se	Cu
T.O	7.74±2.8^{c}	1.81±0.01^{e}	6.12±0.005^{h}	11.71±0.01^{d}	4.89±0.01^{d}	7.68±0.01^{e}	1.55±0.01^{a}	0.55±0.01^{a}	2.13±0.05^{d}
T.O(F)	9.11±0.01^{bc}	1.83±0.01^{d}	5.84±0.005^{g}	11.0±0.01^{e}	4.61±0.01^{f}	6.50±0.01^{g}	1.32±0.05^{b}	0.51±0.005^{a}	1.90±0.1^{e}
A.H	10.16±0.05^{ab}	1.61±0.01^{f}	7.01±0.01^{e}	12.3±0.1^{c}	5.32±0.01^{b}	8.12±0.01^{c}	1.33±0.01^{b}	0.45±0.01^{b}	2.96±0.15^{b}
A.H(F)	10.93±0.05^{ab}	2.01±0.005^{b}	8.16±0.1^{b}	13.08±0.07^{a}	5.97±0.06^{a}	9.58±0.02^{a}	1.11±0.01^{c}	0.51±0.005^{a}	3.83±0.11^{a}
V.A	9.87±0.06^{ab}	1.89±0.005^{c}	7.32±0.02^{d}	11.54±0.01^{d}	4.74±0.03^{e}	6.42±0.01^{h}	1.30±0.01^{b}	0.33±0.01^{e}	1.90±0.01^{e}
V.A(F)	11.13±0.57^{a}	2.01±0.01^{b}	8.95±0.15^{a}	12.83±0.06^{ab}	5.09±0.02^{c}	7.79±0.01^{d}	1.42±0.04^{a}	0.43±0.07^{c}	2.00±0.2^{de}
P.M	9.98±0.01^{ab}	1.88±0.01^{c}	6.80±0.01^{f}	10.61±0.01^{f}	4.4±0.01^{g}	6.84±0.04^{f}	1.11±0.04^{c}	0.38±0.005^{d}	1.88±0.1^{e}
P.M(F)	10.60±0.1^{ab}	2.52±0.01^{a}	7.97±0.12^{c}	12.53±0.4^{bc}	5.03±0.07^{c}	8.96±0.06^{b}	1.11±0.02^{c}	0.39±0.01^{d}	2.46±0.01^{c}

Leafy vegetables contain minerals which are important because of their role as pro-enzyme in some of biochemical reactions in the human body [19]. Table 5 indicates the mineral elements of unfermented and fermented vegetables. Fermentation caused the sodium, magnesium, potassium, zinc, iron, copper and selenium contents of all samples except *Telfairia occidentalis* to increase. Higher calcium content was observed for *Vernonia amygdalina* (9.87 mg/100g -11.13 mg/100g) and *Telfairia occidentalis* (7.74 mg/100g-9.11 mg/100g) after fermentation. Mensah *et al.* (2008) showed that the calcium content of *A. cruentus*, *Gryllotalpa Africana,* and *T. triangulare* were 2.05 mg/100 g, 4.13 mg/100 g, and 7.44 mg/100 g, respectively, which were lower than that in our study.

4. Conclusion

In this study, physicochemical properties of four types of Nigerian vegetables were investigated during fermentation and the involved microorganisms were detected. Based on the obtained results, the considerable amount of minerals in the green leafy vegetable remain constant after fermentation, but vitamin C content decreased. It can be concluded that fermentation is an appropriate method to increase the shelf-life of vegetables after harvesting.

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