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In-Vitro Measurement of pH and Antioxidant Capacity during Colonic Fermentation of Selected Underutilized Wild and Edible Beans

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Abstract

Human gut flora-mediated non-digestible fraction of wild edible and common edible was observed for pH at every 6 hours regime. The antioxidant ability was measured up to 18 hours of fermentation with different associated gut microbes. Changes in pH provide an overview of the fermentation process. In the *in-vitro* study of antioxidant activity by DPPH test, anti-oxidants values showed differences, depending on the substrate and microbial fermenters used for fermentation. At first 6 hours interval, it was observed that the wild bean-*Feregede* fermented by *Enterococcus feacalis* exerts the highest antioxidant capacity of 0.0043 Cathechin equivalents. It also exerts lowest antioxidant capacity of 0.0034 Cathechin equivalents after 18 hours fermentation. These data provided preliminary evidence that consumption of beans diet such as the wild bean—*Otili*, *Feregede*, *pakala* and edible bean—*oloyin* is limiting factor to liberation of antioxidant components during the gastrointestinal digestion. Thus, disruption of normal cellular homeostasis by redox signaling may result in the development of various gastrointestinal pathological conditions, including inflammatory bowel diseases.

Keywords

Wild Beans, Gut-Flora, Dietary-Fibers, Fermentation, Antioxidants

1. Introduction

Bean seeds consist of array of edible types, otherwise known as common bean, and non-common wild types, otherwise known as non-edible types. Of the many known of this legume species, few are used extensively today as food [1] [2] [3].

The edible types are known to be used especially in developing countries as a staple of diet, due to their relatively very low cost, high nutritional value and health benefit [4] [5].

Diet has an important influence on the gut metabolite profile, and this can impact the host health. Thus, dietary compounds are of special relevance for improving the body's defences against such diseases [6]. Information about the role of diet has come from many observational studies around the world across cultures and geographical divide [7] [8]. The presence of antioxidants, such as polyphenols (PP), resistant starch (RS) and non-starch polysaccharides (NSP) in cereal legume products, makes these foods good candidates to qualify as functional foods, promoting health benefits [9] [10]. Beans are rich in a type of antioxidant called polyphenols. They fight the effects of free radicals, which are chemicals that affect a wide range of processes in the body, from physical aging to cancer and inflammation. Relatively little is known about the phenolic compounds in dry beans compared to fruit, vegetables, chocolate, wine, and tea [11].

However, despite the relative abundance of bean products in the diet, scanty data are available on the colonic fermentation of their indigestible components. The purpose of this present work is to relate the effects of dietary fiber on health. In this sense, *in vitro* fermentation models have been used to evaluate the production of gut antioxidants, because these outcomes are difficult to measure *in vivo*.

2. Materials and Methods

2.1. Collection of Cultivar

The legumes (beans) used in this work are of two types; Wild-type beans *Sphenostyles stenocarp* (*Otili;* African yam bean), *Cajanus cajan* (Feregede Pigeon pea), *Phaseolus lunatus* (*Pakala;* lima beans) and Edible bean *Phaseolus vulgaris* (*Oloyin;* kidney bean). They are gotten from the farmers in Ado-Ekiti.

2.2. Media

Media used were Nutrient agar, De Man, Rogosa and Sharpe (MRS) agar, Peptone water. 15 grams of peptone powder was dissolved in 1000 ml of distilled water to prepare peptone water. 5 grams of nutrient agar was dissolved in 178.6 ml of distilled water. 5 grams of the MRS agar was dissolved in 75 ml of distilled water to prepare MRS agar. The media from Oxoid (UK) are then sterilized in an autoclave at 15 psi for 15 minutes. Nutrient agar slants were also prepared to store used organisms.

2.3. Extraction of Non-Digestible Fraction

The alkali-catalyzed hydrolysis method as described by Shimin *et al.* [12] was applied to extract the insoluble dietary fiber from the beans sample. The bean samples were prepared by pulverizing using blender. 20 grams of each sample was place in different beakers in quadruplicate followed by the addition of 25 milliliters of ethyl acetate to each sample. After 3 hours, the slurry was washed

with water and dried with hot air at 55˚C overnight. Sodium hydroxide was added at 20 times the volume of the slurry, and the mixture was then centrifuged at 4000 rpm for 15 min. The collected matter was then deposited and washed with water. The insoluble dietary fiber was recovered from the residue after the deposit was washed with 76% ethanol, 95% ethanol and acetone at 4 times the volume of the slurry and dried with hot air at 55˚C overnight. The insoluble dietary fiber content in the final bean samples extract was approximately 40%, while the other 60% of the extract was nitrogen free extract (NFE). The content of IDF was determined according to National Food Safety Standard Determination of Dietary Fibers in Foods (GB 5009.88-2014).

2.4. Isolation of Organisms

Lactobacillus acidophilus was isolated by Streak Plate Method from milk gotten from Ayetoro Ekiti using the MRS agar (Oxoid, UK). The other microorganisms (*Enterococcus feacalis*, *Escherichia coli*, *Streptococcus pyogens*, *Staphylococcus aureus* and *Bacillus subtilis)* used were obtained from the Microbiology Laboratory, Ekiti state University, Ado Ekiti by Serial Dilution. One loopful from the stocks was dispensed into 9 ml of distilled water and serial diluted in dilution 10^{-1} to 10^{-7}. A loopful was then inoculated into the MRS agar, nutrient agar and peptone water. From the pure culture a loopful were inoculated into the nutrient slant for preservation.

2.5. *In Vitro* Gastrointestinal Fermentation

In vitro colonic fermentation Total Indigestible Fraction isolated from the bean was fermented in disposable test tubes prepared with peptone water under strict anaerobic conditions, at 37˚C (13). A 1:10 (w/v) dilution of selected gastrointestinal microbes (*Escherichia coli*, *Bacillus subtilis*, *Lactobacillus acidophilus Enterococcus feacalis*, *Streptococcus pyogene* and *Staphylococcus aureus*) with 0.1 mol/L, pH 7 phosphate buffer was prepared and homogenized in a digital high-speed homogenizer system (IKA-Ultra-Turrax T18, USA). The resulting suspension (1 ml) was distributed in disposable test tubes (containing 9 ml of peptone water), and 0.1 g of the isolated total indigestible fraction from each bean was added. All incubations were performed in triplicate, and the corresponding tubes from samples and controls were analyzed for pH changes, turbidity at each fermentation time point. Each tube was mixed with 100 µL of Sodium hydroxide at room temperature to stop the reaction. The tubes obtained at each time of fermentation were centrifuged (Hermle Z 323 K; Wehingen, Germany) at 3500 9 g for 15 min at 4˚C. Supernatants were divided into two parts: one was used for metabolite profile analysis (short chain fatty acids), and the other was used for Radical assays and identification of the glucose concentration. Samples were always kept at −80˚C until analysis.

2.6. Determination of pH

At each fermentation point (0 h, 6 h, 12, 18 h, 24 h) , the pH was measured using

pH meter.

2.7. Determination of Antioxidant Activity by the DPPH Test

Reagent preparation: 0.95 mmolL^{-1} solution of radical DPPH (m = 0.00374 g/100 ml). First, this amount is dissolved in 50 ml of DMSO and after dissolution made up to a volume of 100 ml with ACS water. The solution can be used for 7 days when stored at 4˚C and in the dark. Measurement procedure for an automated analyzer: A 200 µl volume of reagent is incubated with 20 µl of sample (gallic acid). Absorbance is measured after 1520 seconds at λ = 510 nm.

3. Results and Discussion

Fermentation studies of pH and antioxidant activity had been observed in order of increasing level of different microbial fermenters by the samples. It was observed that various gastrointestinal tract microbe fermenters such as *E. coli, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus feacalis and Lactobacillus acidophilus* were found increasing per interval as stated. At the start (**Figures 1-4**), the pH value was 7.0 approximately before fermentation was increased. At 6 hrs, fermenter microbe in *otili* such as *E. coli* had the highest value pH of 14.0 (**Figure 4**) followed by *pakala* such as *E. coli* having 12.0 pH (**Figure 1**) value while the remaining microbes fermenters (**Figure 2** & **Figure 3**) had at least pH of 8.0 expect from the microbe fermenter in *pakala* such as *Streptococcus pyogenes* having 7.0 pH value. At 18 hrs, fermenter microbe in *otili* (**Figure 4**) had the highest increasing value pH of 14.0 while microbe fermenter in *oloyin* (**Figure 2**) had the least pH value of 6.0 - 6.5. At 24 hrs, all the fermenters microbes (**Figures 1-4**) had pH values 6.0 to 8.0. It is suffice to submit that changes in pH provide an overview of the fermentation process (**Figures 1-4**). Decreases in the pH units during fermentation of the Bean samples indicate the potential and beneficial action of these products on

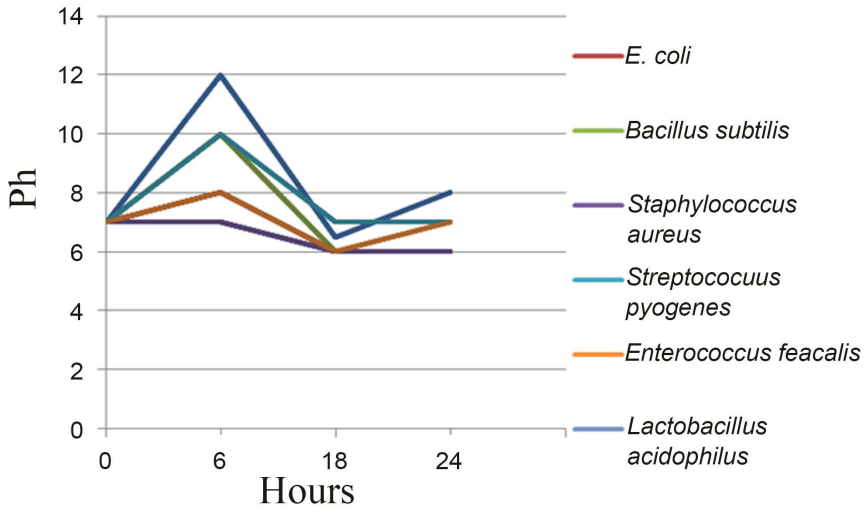

Figure 1. pH changes during colonic fermentation of *Pakala*.

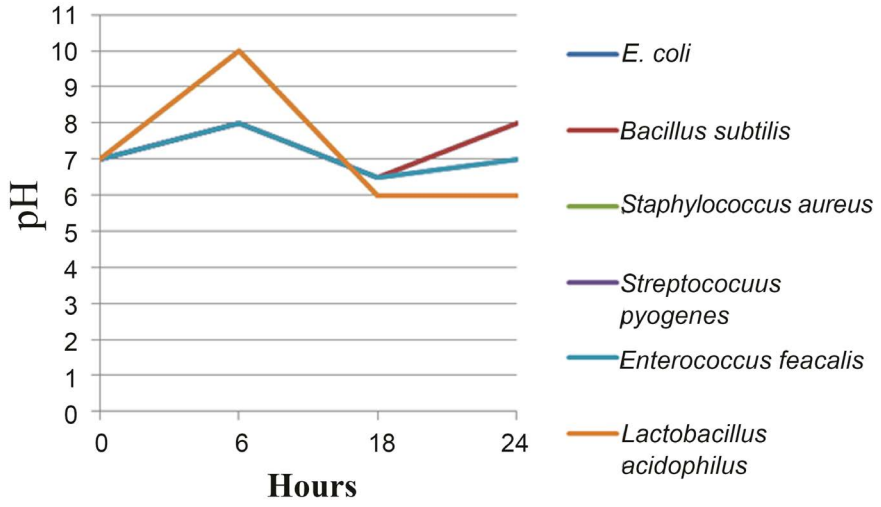

Figure 2. pH changes during colonic fermentation of *Oloyin*.

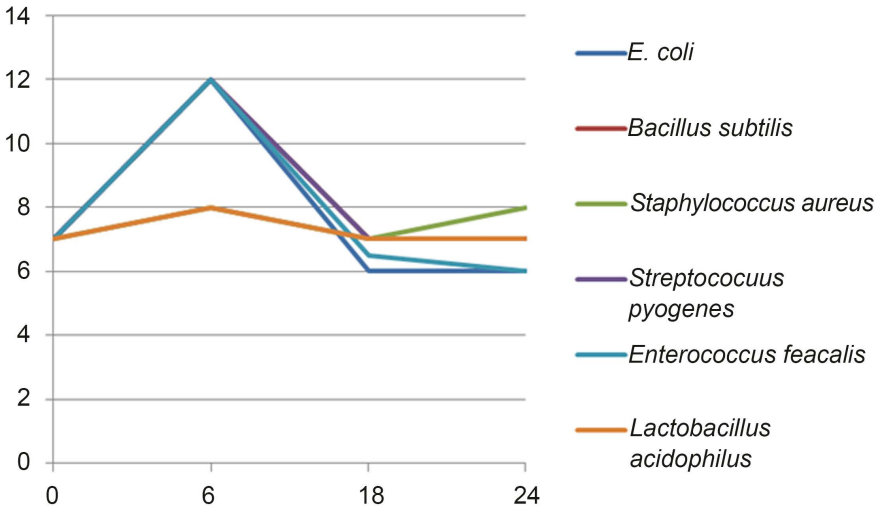

Figure 3. pH changes during colonic fermentation of *Feregede*.

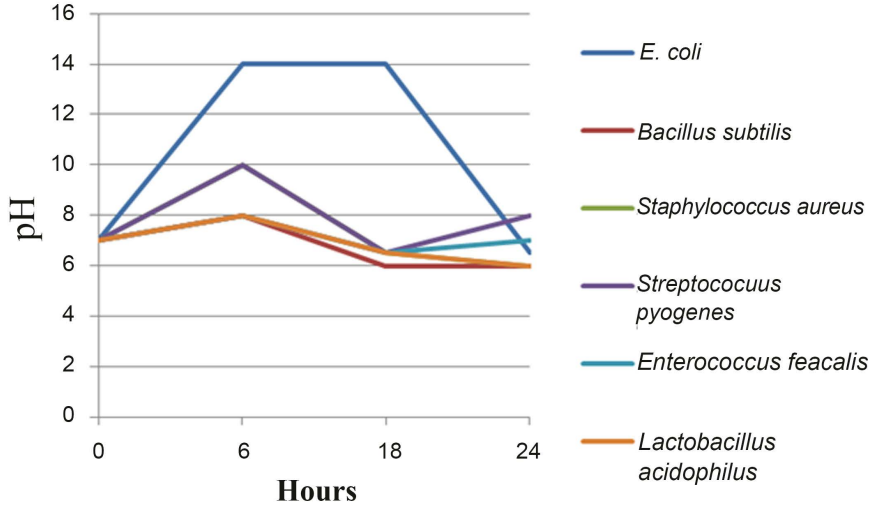

Figure 4. pH changes during colonic fermentation of *Otili*.

the host health [13] [14] [15]. The resulting pH suggests that gut microbiota composition have the ability to prevent overgrowth of pH-sensitive pathogenic bacteria, such as *Entero bacteriaceae* and *Clostridia spp* [16] [17] [18] [19].

In the *in-vitro* study of antioxidant activity by DPPH test, antioxidants values (**Figures 5-8**) showed differences, depending on the substrate and microbial fermenters used for fermentation. At the second 6 hour regime, it was observed that the wild bean—*Feregede* fermented by *Enterococcus feacalis* and *Bacillus subtilis* exert the highest antioxidant capacity of 0.0043 Cathechin equivalents (**Figure 7**). Similar result was also found during fermentation of *Oloyin* by *Lactobacillus acidophilus* (**Figure 6**). The lowest antioxidant capacity of 0.0036 Cathechin equivalents recorded in the course of the entire experiment was found

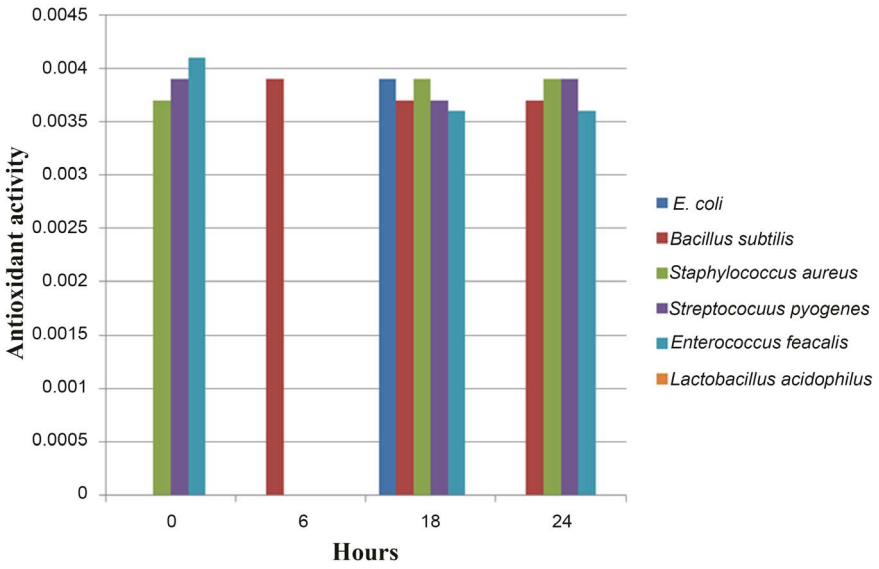

Figure 5. Antioxidant activity during colonic fermentation of *Pakala*.

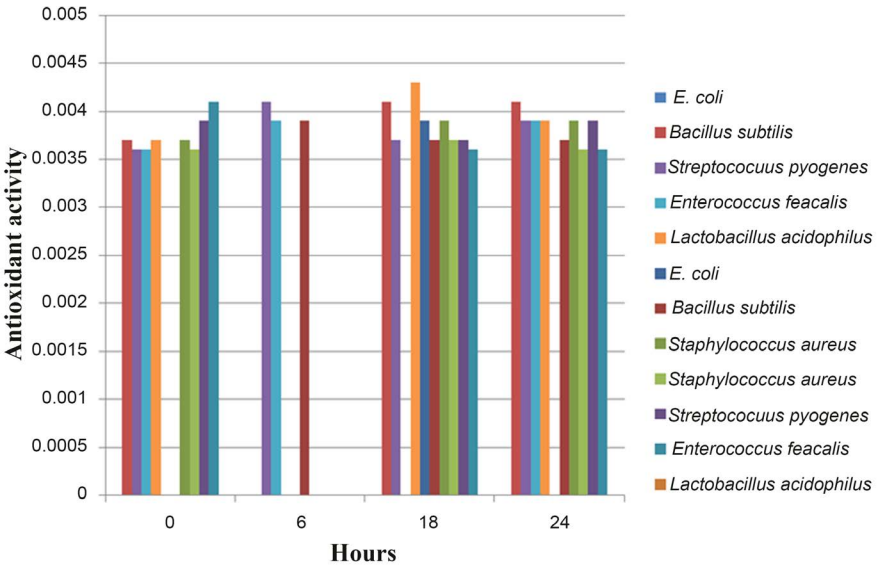

Figure 6. Antioxidant activity during colonic fermentation of *Oloyin*.

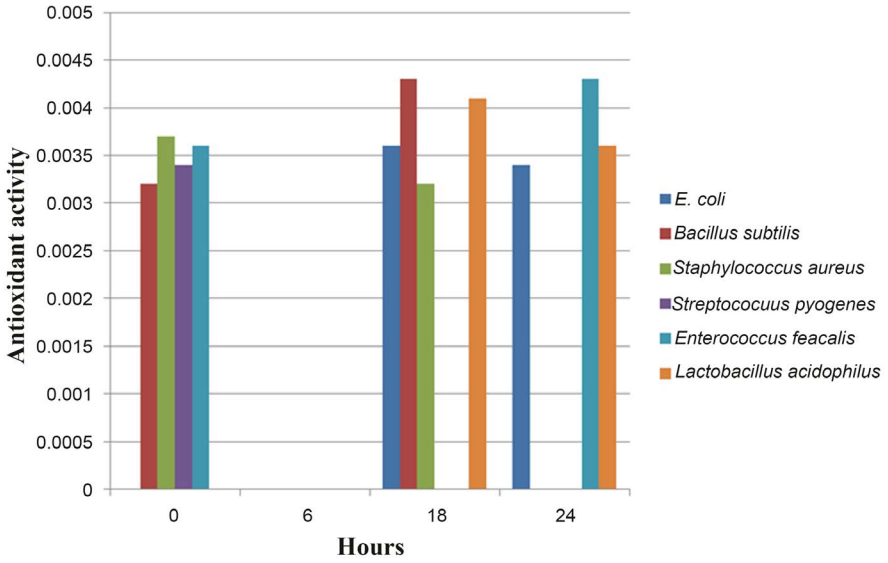

Figure 7. Antioxidant activity during colonic fermentation of *Feregede*.

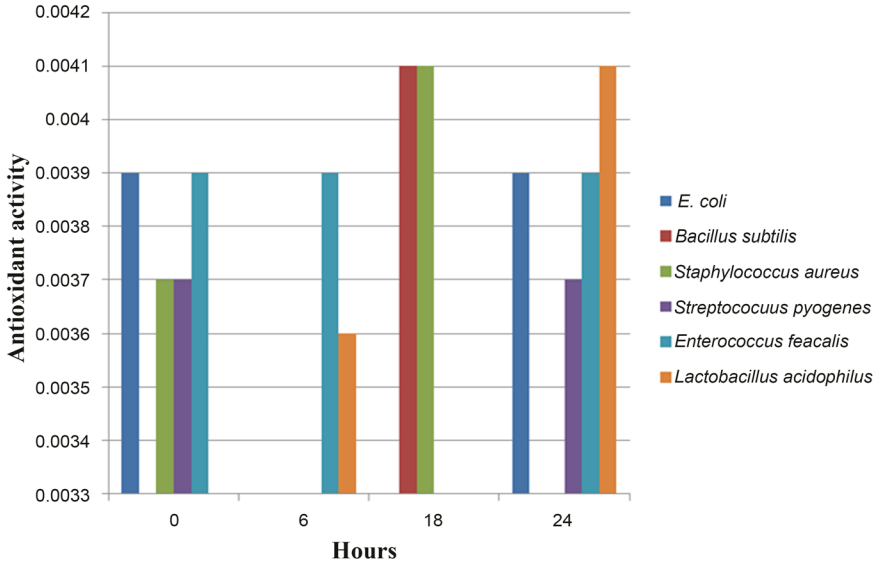

Figure 8. Antioxidant activity during colonic fermentation of *Otili*.

after 6 hour fermentation of *Lactobacillus acidophilus* on *Otili* (**Figure 8**). Campos-Vega *et al.* [20] submitted that antioxidant activity of spent medium or dark roasted coffee are affected by pH changes during *in vitro* gastrointestinal digestion and colonic fermentation, probably modifying their physicochemical properties (*i.e.* chemical structure and solubility), suggesting their potential health benefits. Additional studies suggest that *in-vitro* antioxidant action of foods is limited to liberation of antioxidant components during the gastrointestinal digestion [21]. However, *in-vivo* evidence suggests that disruption of normal cellular homeostasis by redox signaling may result in the development of various gastrointestinal pathological conditions including gastro-duodenal ulcers, gastrointestinal cancer, and inflammatory bowel disease [22]. Furthermore, the in-

crease in dietary fiber present in a food can stimulate the fermentative production of butyric acid and this metabolite may also exert *in vitro* and *in vivo* antioxidants by the increase in glutathione production and decreasing the formation of reactive oxygen species [23] [24].

4. Conclusion

Beans are good sources of antioxidants compounds and this research provides information of their availability, mainly for non-digestible dietary fiber of beans on gastrointestinal tract associated microbes.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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