

Profile of Turbidity and Glucose Formation from Underutilised Wild, Edible Bean during *In-Vitro* Gastro Intestinal Digestion and Fermentation

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Abstract

Fermentation takes place throughout the gastrointestinal tract of all animals, but the intensity and products of fermentation depend on number and types microbes, which are generally highest in the large bowel. Large intestinal epithelial cells do not produce digestive enzymes, but contain huge numbers of bacteria which have the enzymes to digest and utilize many substrates. The seeds of beans (*Otili*, *Feregede*, *Pakala* and *Oloyin*) analyzed in this present study contain indigestible fraction called dietary fiber which helps to maintain functioning of the digestive system. Fermentation of indigestible fraction (IF) of these beans was mimicked through *in-vitro* method which leads to biochemical changes in the samples. During this experiment, increase in acidity and turbidity was observed. The glucose concentration decreases with some exceptions, such as *Pakala* fermented by *Lactobacillus acidophilus* which had the value of 6.260 mmol/L at 6 hr and increased to 6.616 mmol/L after 18 hours fermentation, *Otili* fermented by various microorganisms which had its turbidity increased by 50%. *Lactobacillus acidophilus* fermenting *Pakala* had the highest glucose concentration during the fermentation period. The increase in turbidity could be as a result of increase in microbial flora or production of metabolites, such as glucose. The approach followed here may be used as a predictive model to assess the metabolic implications of food substrates present in the traditional Nigerian orphan beans.

Keywords

Fermentation, Turbidity, Glucose, Indigestible-Fraction, Wild-Bean

1. Introduction

Prebiotics are non-digestible but fermentable oligosaccharides that are specifically designed to change the composition and activity of the intestinal microbiota aimed at promoting the health of the host. Dietary fiber and non-digestible oligosaccharides are the main growth substrates of gut microorganisms. The bacterial population of the gut has been studied in diseases, such as colon cancer, inflammatory bowel diseases (IBD), hypercholesterolemia, non-alcoholic fatty liver disease (NAFLD) and others [1] [2].

Microbial metabolic end-products, which account for one third of the metabolites present in the human blood, play an important role in gut homeostasis and have an impact on host metabolism and health [3] [4] [5] [6] [7]. Non-digestible oligosaccharides are considered non-caloric agents because of their resistance to the hydrolytic action of digestive enzymes [8] [9]. Oligosaccharides are excellent prebiotics, because they act like growth factor to particular commensal bacteria, which inhibit the adherence and invasion of pathogens in the colonic epithelia by competing for the same glycoconjugates present on the surface of epithelial cells, altering the colonic pH, favoring the barrier function, improving the mucus production, producing short-chain fatty acids and inducing cytokine production, promoting multiple benefits and better health [10] [11] [12]. These prebiotics effects can be evaluated on the basis of growth of bacteria, such as lactobacilli and *bifidobacteria*, the decrease of intestinal pathogens and the increase or decrease in production of health related bacterial metabolites [2] [13].

The ultimate aim of supplementation of the human diet with prebiotics is the beneficial management of the gut microbiota [5] [8]. Many non-digestible oligosaccharides beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon, thereby improving host health [14] [15]. Also, it has been found out that changes in the microflora of gastro-intestine as a result of ingesting non-digestible oligosaccharides increase the protection of the human intestine by reducing the antagonist effects of pathogenic bacteria, thereby decreasing the intestinal infections, diarrhoea disease, stimulating the immune system and antigenotoxicity activities [16] [17] [18].

Interest in functional oligosaccharides is motivated by their diverse industrial applications in nutraceutical, pharmaceutical, prebiotics, cosmetics, animal feed and agriculture sector. The health benefits of functional oligosaccharides are well-known and far-reaching. Furthermore, oligosaccharides have been the subject of intensive and cutting-edge research during the last three decades in response to their ever-increasing demand [19] [20] [21] [22] [23]. To this end, several studies have focused their efforts on investigating the effect of some in the growth of intestinal microbiota [24] [25] [26]. Past and recent studies suggest that one can positively modify the contents of the gut microbiota by introducing prebiotics, probiotics, synbiotics and other therapeutics. Hence, this paper focuses on prebiotic tendency of under-utilized wild bean compared to a common edible bean and their possible inclusion of these wild underutilized to our diet.

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 are; Nutrient agar, 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 are then sterilized in an autoclave at 121 °C, 15 Pascal 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.*, [27] 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 placed 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 GB 5009.88-2014.

2.4. Isolation of Organisms

Lactobacillus acidophilus was isolated from milk gotten from Ayetoro Ekiti using the MRS agar. The other microorganisms (*Enterococcus faecalis*, *Escherichia coli*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Bacillus subtilis*) used were obtained from the Microbiology Laboratory, Ekiti state University, Ado Ekiti. One loopful from the stocks was dispensed into 9ml of distilled water and serially 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 [28]. A 1:10 (w/v) dilution of selected gastrointestinal microbes (*Escherichia coli*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, *Streptococcus pyogenes* 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; 1 min, 7847 g). 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 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 the Turbidity

During fermentation, the absorbance level was assessed at 0, 6, 12 and 24 h as a function of the fermenting medium turbidity. The absorbance was read at 540 nm via Camspec M106 Spectrophotometer (USA).

2.7. Glucose Test (GT)

Using commercially available Randox Kit 100 µl of the supernatant and 100 µl of the reagent was mixed and incubated for 25 minutes at 15°C to 25°C. The absorbance of the standard (A_{standard}) was measured and the sample (A_{sample}) against the reagent blank within 60 minutes.

3. Results and Discussion

The seeds of beans (*Otili*, *Feregede*, *Pakala* and *Oloyin*) analyzed in this present study contains indigestible fraction called dietary fiber. The result of the fermentation process on introduction of several microorganisms involves change in turbidity and glucose level.

Fermentation occurring in the mono gastric gastrointestinal tract (GIT) is increasingly being recognized as having an important influence on health both of the GIT itself, and also of the host animal. From the point of view of GIT health, it is recognized that fermentation is important for gut motility, the improvement of energy yield, the production of vitamins, and the stimulation of gut immunity [29]. **Figures 1-4** show the absorbance level during the fermentation period. Generally, the absorbance level increases as the fermentation period increases, implying that the population of each microorganism increases. *Pakala* fermented by *Streptococcus pyogenes* had the highest absorbance of 1.997 after 24 hours of

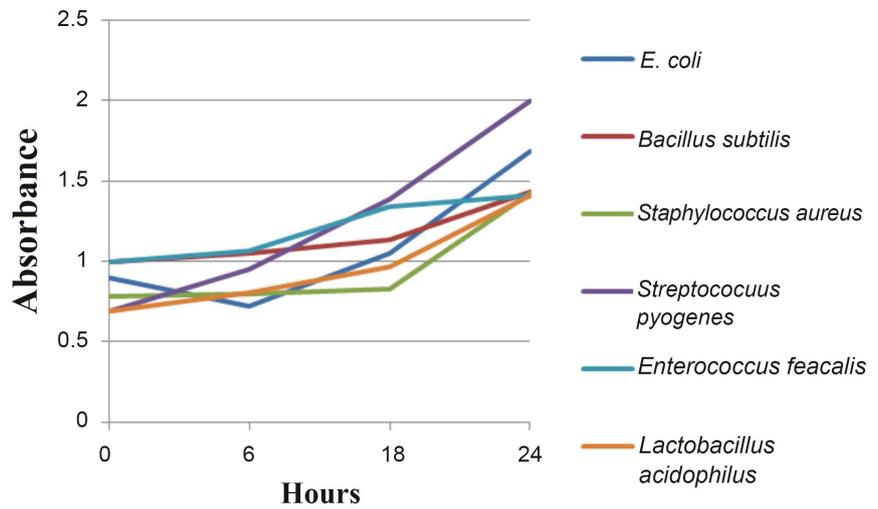


Figure 1. Absorbance of fermented Pakala.

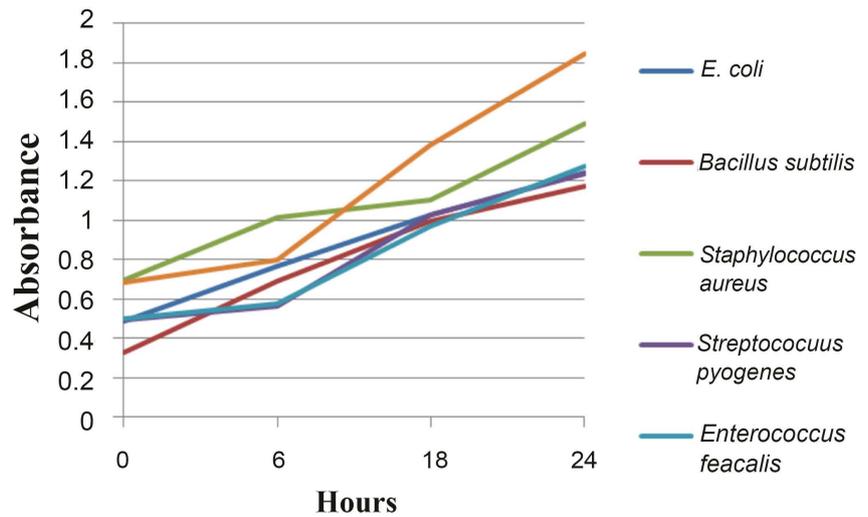


Figure 2. Absorbance of fermented Oloyin.

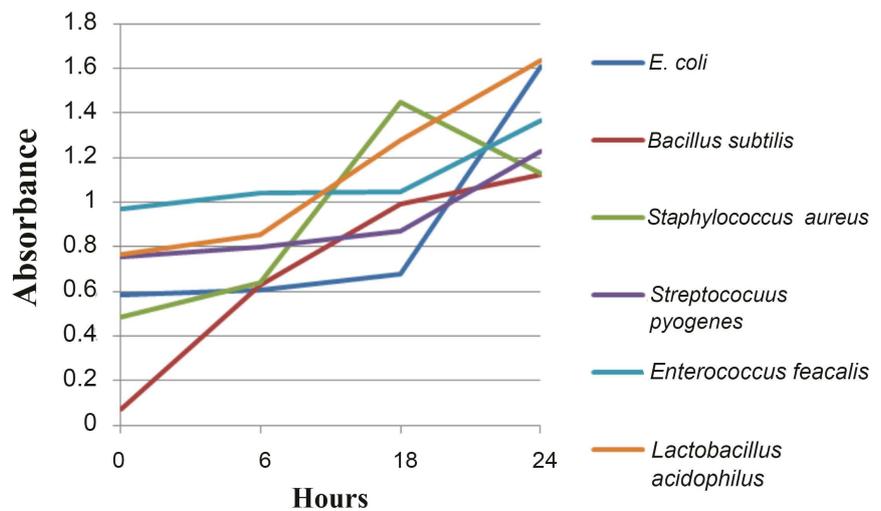


Figure 3. Absorbance of fermented Feregede.

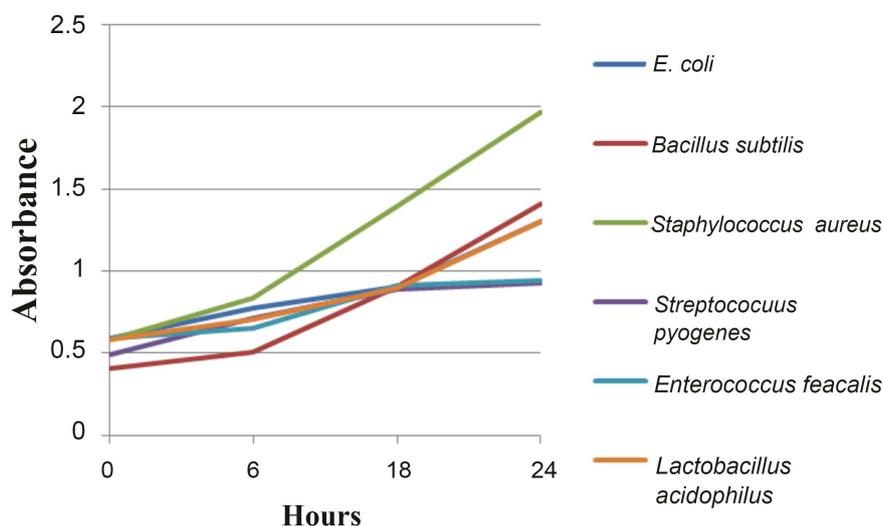


Figure 4. Absorbance of fermented *Otili*.

fermentation having a relative difference to that of *Otili* fermented by *Staphylococcus aureus* (1.969). At 0hr, *Pakala* has the highest absorbance, *Feregedede* as the next followed by *Oloyin* and *Otili*. After 24 hours of fermentation, *Otili* fermented by various microorganisms had its absorbance increased by 50%. *Pakala* fermented by *Streptococcus pyogenes* had the highest absorbance of 1.997 after 24 hours of fermentation with a relative difference to that of *Otili* fermented by *Staphylococcus aureus* (1.969). In this study, the absorbance level increases as the fermentation period increases, implying that either the population of each microorganism increases or their respective metabolic products [16] [30].

From the absorbance level during the fermentation period, the turbidity level was observed as the microbial fermenters were increased per interval hours of 0 hr, 6 hrs, 12 hr, 18 hrs and 24 hrs. At 0hr, microbial fermenters in *pakala* had the highest increasing level of 0.689 - 0.897 while *otili* falling arise to 0.408 - 0.590 in an increased level. At 6hrs, 0.718 - 1.061 was obtained in microbes fermenters in *pakala* while microbes fermenters in *otili* had the least level of 0.507 - 0.838. At 18 hrs, all the microbial fermenters had a minimum increasing level of absorbance. At 24 hrs, all the microbial fermenters had a minimum increasing level of absorbance except from *otili* which had its absorbance increased by 50%.

Glucose test was used to examine glucose homeostasis during fermentation period (Figures 5-8). The glucose concentration for *Oloyin* fermented by *Bacillus subtilis* increased from 1.327 mmol/L (0 hr) to 4.795 mmol/L after 24 hr. *Lactobacillus acidophilus* fermenting *Pakala* had the highest concentration of glucose (6.616 mmol/L) followed by *Otili* fermented by *Enterococcus faecalis* (5.861 mmol/L). No difference was found in both *Feregedede* fermented by *Lactobacillus acidophilus* and *Otili* fermented by *Escherichia coli*. From the result of the glucose of fermentation extract, *Pakala* fermented by *Lactobacillus acidophilus* had the highest concentration of glucose (6.616 mmol/L) followed by *Otili* fermented by *Enterococcus faecalis* (5.861 mmol/L). The glucose concentration

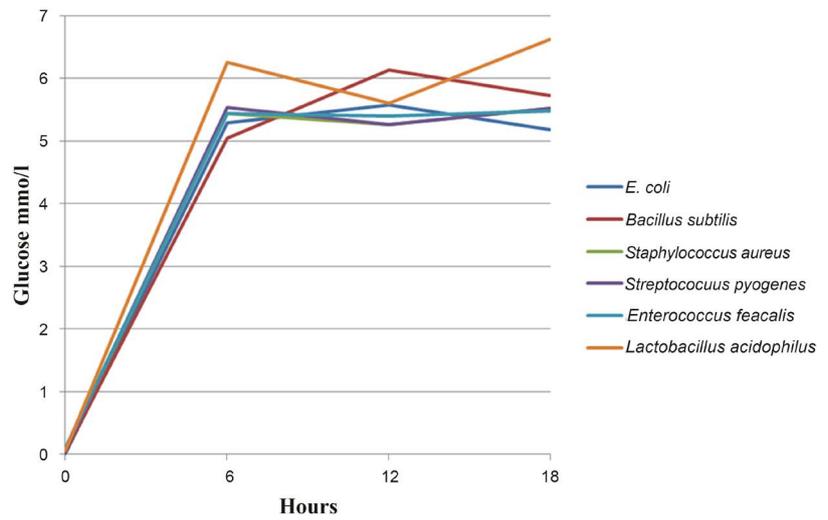


Figure 5. Glucose formation during fermentation of dietary fibre from *Pakala*.

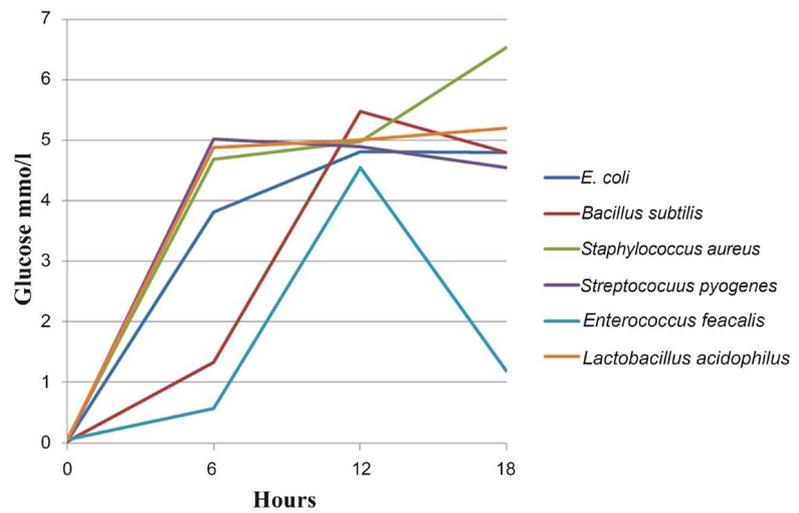


Figure 6. Glucose formation during fermentation of dietary fibre from *Oloyin*.

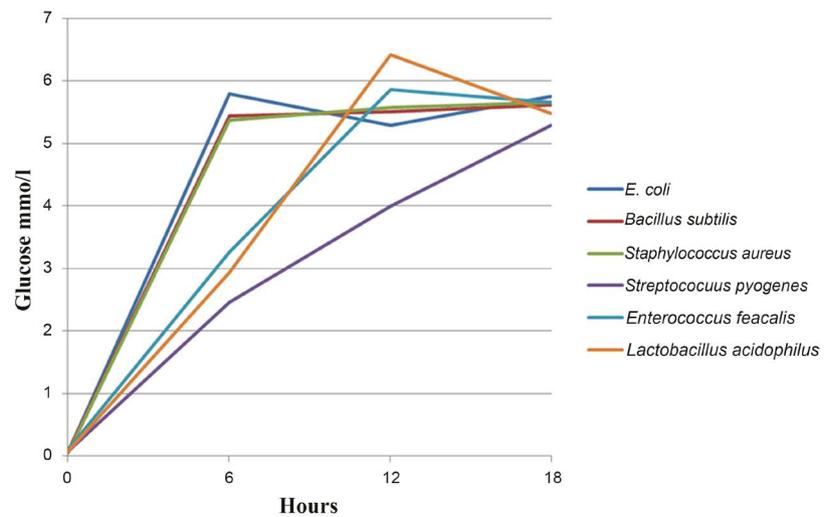


Figure 7. Glucose formation during fermentation of dietary fibre from *feregede*.

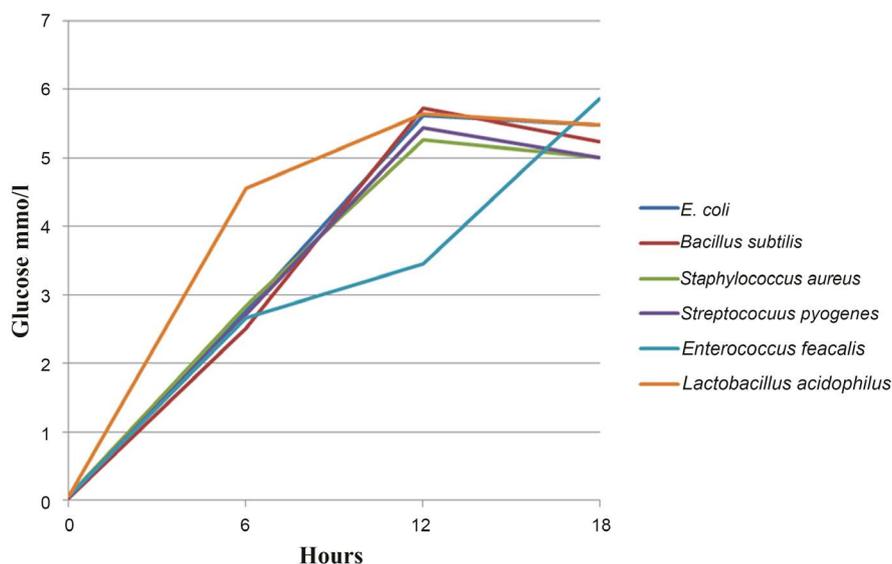


Figure 8. Glucose formation during fermentation of dietary fibre from *feregede*.

decrease with some exception such as *Pakala* fermented by *Lactobacillus acidophilus* which had the value of 6.260 mmol/L at 6hr and increased to 6.616 mmol/L after 18 hour. Probably the microbes *Lactobacillus acidophilus* and *Enterococcus faecalis* (*Otili*) having high glucose concentration may be as a result of high metabolic activity thus converting the oligosaccharide into simple sugar such as glucose for their growth. The ability of this organism to produce more glucose is explained by its higher affinity for the substrate [31] [32] [33].

4. Conclusion

This contribution shows the changes of microbiota metabolites, such as glucose as influenced by change in turbidity and pH during *in vitro* colonic fermentation of indigestible fraction isolated from three underutilised wild Nigerian beans. The magnitude of metabolite production, particularly glucose, during *in vitro* fermentation, was dependent on fermentation time and types of substrate. It should be noted that, through the study of foods frequently consumed by a population, it will improve our understanding of the effects of diet on colon health promotion.

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Conflicts of Interest

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

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