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Article

Fermented Rice Beverages Modulate Key Enzymes Related to Type 2 Diabetes *in vitro*

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Abstract: Background: The therapeutic benefits of fermented foods in the treatment and prevention of Type 2 diabetes mellitus have been reported. Aim: Inhibitory effect of fermented rice beverages on α-amylase, α-glucosidase and pancreatic lipase was evaluated. Method: Two fermented rice beverages-fermented rice alone (FKR) and fermented rice plus roasted peanuts (FKRG) were produced using spontaneous fermentation. Capacity of the beverages to inhibit alpha-glucosidase, alpha-amylase and pancreatic lipase *in vitro* was evaluated and compared with standards (positive controls). Results: FKR exerted inhibition of α-glucosidase between 9.23-21.11% and FKRG 1.11-17.36% at the various concentrations respectively, with both samples showing the most significant inhibition (about 20%) at 125 µg/mL. FKRG exhibited greater alpha-amylase inhibition activity than FKR, but for both samples, the most significant ($P < 0.05$) inhibition occurred at 500 µg/mL. With pancreatic lipase, no significant inhibition was observed for both FKR and FKRG at the tested concentrations compared to Orlistat used as control; however, at 31.25 µg /mL FKRG showed an inhibitory effect of approximately 15%, which was not evident at higher concentrations. Conclusion: Low to moderate inhibition of α-amylase, α-glucosidase and pancreatic lipase by both FKR and FKRG, showed that the fermented rice beverages have potential to modulate hyperglycemia in type 2 diabetes *in vitro*. This is an indication that fermented rice beverage could prevent postprandial hyperglycemia *in vivo*.

Keywords: fermented beverages; type 2 diabetes; postprandial hyperglycemia; carbohydrate metabolizing enzymes

1. Introduction

Fermentation of foods is an ancient science used to process and preserve foods. The products of fermentation have been found to possess great nutritional and therapeutic benefits for humans. This accrue from the breakdown and conversion of food components resulting in improved food digestibility and nutrient availability, detoxification and degradation of anti-nutrients, synthesis and increased availability of Vitamin B12, niacin, provision of prebiotic and probiotic components [1].

Increasingly, fermented foods and beverages are being researched for their health benefits in preventing, treating or managing chronic diseases. Fermented foods and beverages produced by the metabolic activity of microorganisms such as bacteria, yeast, and fungi have gained attention for their potential health benefits, including for individuals with diabetes [2]. Several studies have reported the health benefits of fermented foods and beverages, which include enhancement of gut microbiota, reducing cardiovascular disease risks, antioxidant, antimicrobial, anti-inflammation, anti-obesity, anti-cancer, anti-hypertensive, enhancing insulin secretion and immune-modulatory activities [2-6].

Fermented beverages remarkably, are increasingly gaining recognition as functional foods, especially with therapeutic benefits against chronic diseases. Many fermented beverages such as "kunu" (fermented millet) "kefir" (fermented milk), "kombucha" (fermented tea), beer, wine, etc,

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exist around the world [6-8] and have been reported to mitigate the incidence of several chronic conditions [4,9-10].

Diabetes is a chronic metabolic disorder characterized by high blood glucose levels due to insulin resistance or insufficient insulin secretion. According to IDF [11] reports, over 537 million people currently live with diabetes globally, with a projection that this number will increase to 700 million by 2045.

Studies have shown that fermented foods can improve gut microbiota composition and function, potentially reducing diabetes risk. For example, a study by Naruszewicz et al. [12] found that daily consumption of fermented milk containing Lactobacillus fermentum LF15 and Lactobacillus plantarum 299v significantly reduced LDL cholesterol and triglycerides in patients with type 2 diabetes. Another study by Kim and Shin [13] found that water-soluble chicory extracts in fermented beverages reduced glucose uptake in rats.

Fermented beverages may also have anti-inflammatory and antioxidant properties, capacity to improve insulin sensitivity and reduce diabetes risk. Xiao et al. [14] found that rats fed a cinnamon extract diet had improved insulin sensitivity and reduced inflammation. However, inter-individual variability in gut microbiota composition and response to dietary interventions may limit the generalizability of these findings.

The effects of fermented foods and beverages on glycemic control and other diabetes markers have been reported. For instance, Nilsson et al. [15] found that a fermented oatmeal drink resulted in a significantly lower glycemic response in healthy subjects. Additionally, a fermented milk drink improved insulin sensitivity and reduced fasting blood glucose levels in individuals with type 2 diabetes [16]. These studies suggest the benefits of fermented beverages for glycemic control and insulin sensitivity in individuals with diabetes.

However, the potential mechanisms underlying the beneficial effects of fermented beverages on diabetes are not fully understood. Fermentation may increase the bioavailability and bioactivity of certain nutrients like peptides and polyphenols, which might have antidiabetic effects [14]. These bioactivities are attributed to the influence of the fermenting microorganisms on the gut microbiota, which then mediate the effects of fermented foods and beverages on glycaemic control, regulate appetite and food intake, reduce inflammation and inhibit carbohydrate metabolizing enzymes [12,16-18].

Rice (Oryza sativa) in various forms (polished, brown, white, etc) is a staple cereal grain food in many diets all over the world [19-20]. Rice is not often recommended for diabetics because of its high glycemic index which tends to increase the risk of diabetes [21-22]. However, fermentation is known to increase nutrient content and remove/decrease anti-nutritional factors of many cereal grains [23].

In view of the increasing consumer demand for functional foods, a non-conventional, non-alcoholic beverage was prepared from polished rice grains, spices and groundnuts, simulating a traditional, Nigerian millet-based beverage ("Kunu").

The aim of this study was to assess the potential of fermented rice beverages to inhibit alpha-glucosidase, alpha-amylase and pancreatic lipase in vitro. This is to the end that consuming rice in a different form may have functional anti-diabetic benefits through inhibition of the above mentioned enzymes.

2. Materials and Methods

This was an *in vitro* study invovling fermented rice beverages: fermented rice alone (FKR) and fermented rice plus roasted peanuts/groundnuts (FKRG) produced using spontaneous (native flora) fermentation. Inhibitory capacity of the freeze-dried extracts against alpha-glucosidase, alpha-amylase and pancreatic lipase were then evaluated and compared against standards (positive controls). The standards used were- Epigallocatechin gallate (EGCG 200 μM) for alpha-glucosidase, Acarbose [500 μM] for alpha-amylase, and Orlistat (50 μM) for pancreatic lipase respectively.

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Reagents

All reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and freshly prepared for the assays. Buffer: 67 mM potassium monobasic anhydrous phosphate pH 6.8, 3 mM reduced glutathione, p-NP-Gluc: 10 mM p-Nitrophenyl α-D-glucopyranoside, Na_2CO3: 100 mM sodium carbonate, Enzyme: 50 µg/mL α–glucosidase from Saccharomyces cerevisiae, Positive control: Epigallocatechin gallate (EGCG), Dulbecco's Phosphate Buffered Saline (PBS), porcine pancreatin (1 mg/mL) in PBS, Starch solution: 2 mg/mL in distilled water, Stop solution: 1 M HCl in distilled water, Iodine reagent: 0.127 g iodine; 0.083 g potassium iodide in 100 mL distilled water, Positive control: Acarbose.

Preparation of Fermented Rice Beverage (Kunu)

The fermented rice beverage was prepared as for Nigerian fermented millet beverage (Kunu) [24] with modifications. Briefly, rice (250 g in 1000 mL tap water), was soaked and allowed to spontaneously ferment (native fermentation) for 48 h after which 5 g of ginger, 3 g cloves and 1 g cayenne pepper were added. It was ground into a smooth paste using a laboratory blender (Waring Commercial, Torrington, CT 06790), then sieved using a muslin cloth. The sieved slurry was allowed to settle for 3 h after which the clear liquid was decanted. The slurry was divided into two equal halves, half of the thick slurry was put in a medium sized bowl and boiling hot water (about 500 mL), was added to it and mixed to give a porridge/pap-like product. The remaining half was then added to this and mixed thoroughly to produce the rice 'kunu'-this is the fermented rice kunu (FKR). The fermented rice plus groundnut beverage was prepared following the same procedure as described, except that 50 g of roasted peeled groundnuts, soaked for 1 h was added to the fermented rice just before grinding. This is the fermented rice kunu with groundnuts (FKRG).

The fermented rice beverages were stored in glass bottles at 4°C in the refrigerator. One hundred (100 mL) of each beverage was taken, filtered under pressure using a Buckner funnel and filter paper (Whatman No. 1) and freeze-dried to obtain samples for analyses.

Sample preparation

Test samples (fermented rice beverages) were reconstituted in dimethyl sulfoxide (DMSO) to a final concentration of 100 mg/mL. Samples were diluted serially in assay buffer to concentrations of 500, 250, 125, 62.5 and 31.25 µg/mL.

Alpha-glucosidase inhibition assay

Into a 96 well micro-titer plate, 10 µL of sample and 70 µL enzyme were added and incubated at 37°C for 10 minutes. After this, 20 µL p-NP-Gluc was added and incubated at 37°C for 20 minutes. Finally, 25 µL Na_2CO_3 was added to the mixture, then Absorbance was measured at 410 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). No enzyme and no substrate controls were included and the percentage α-glucosidase inhibition was calculated as:

$$\%\ \alpha\text{–}glucosidase\ inhibition = \frac{(\mathbf{A410nm\ of\ control - A410nm\ of\ test\ sample})}{\mathbf{A410nm\ of\ control}}\ x\ 100$$

All the assays were in triplicates.

Alpha-Amylase inhibition assay

The α-amylase inhibition assay was also performed in a 96-well micro-titer plate in quadruplicate. To the 96-well plate, 15 µL sample and 5 µL of enzyme were incubated for 10 min at 37°C. Then 20 µL starch solution was added, incubated for 30 min at 37°C, after which the reaction was quenched with 10 µL stop solution and 75 µL iodine reagent was added. Absorbance was measured at 580 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). No enzyme and no substrate controls were included, and the percentage α-amylase inhibition was calculated as:

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$$\%\ \alpha\text{–}amylase\ inhibition = \frac{(\text{amylase activity of control} - \text{amylase activity of test sample})}{\text{amylase activity of control}}\ x\ 100$$

where $amylase\ activity = A580nm\ without\ enzyme - A580nm\ with\ enzyme.$

Lipase inhibition assay

The pancreatic lipase inhibition assay was performed as described by Pringle *et al.* [26]. Briefly, 10 µL sample plus 5 µL enzyme were incubated at 37°C for 15 minutes. Then 170 µL of substrate with reaction buffer were incubated at 37°C for 25 minutes. The absorbance was measured at 405 nm using a BioTek® PowerWave XS spectrophotometer (Winooski, VT, USA). No enzyme and no substrate controls were included. Orlistat (50 µM) was used as a positive control and the percentage lipase inhibition was calculated as:

$$\%\ Lipase\ inhibition = \frac{(\text{A405nm of blank} - \text{A405nm of test sample})}{\text{A405nm of blank}}\ x\ 100$$

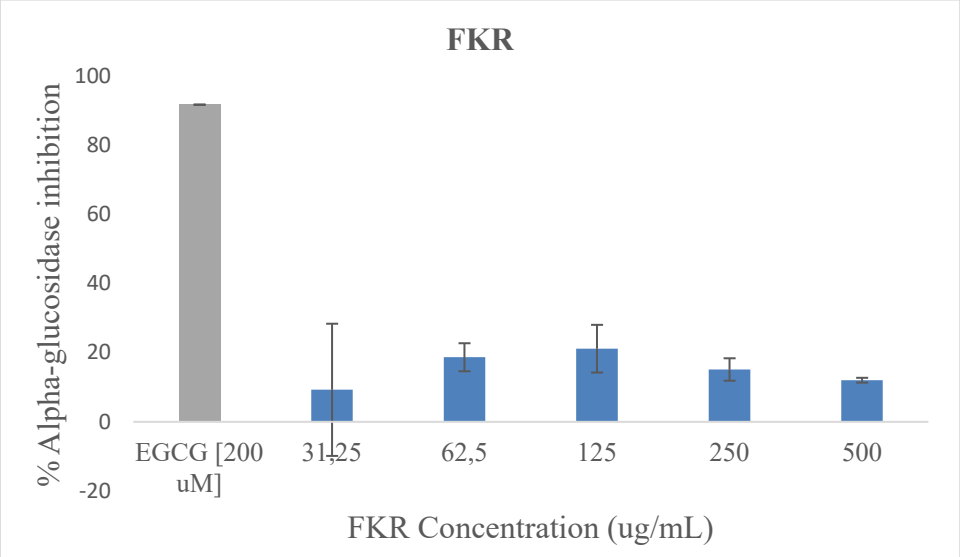
Statistical Analysis

All assays were performed in triplicates and data were expressed as mean ± standard deviation (SD), using one way analysis of variance (ANOVA) and Fischer's Least Significant Difference on the MINITAB 17 statistical software package. Mean values were regarded as significantly different at $P < 0.05$.

3. Results

3.1. Alpha-glucosidase inhibition

Alpha-glucosidase inhibition capacity of the samples is shown in Figure 1. The two fermented rice beverages (FKR and FKRG) exhibited modest alpha-glucosidase inhibition activity, though these were considerably low compared to EGCG which was used as the standard/positive control. FKR exerted inhibition of α-glucosidase at a range of 9.23-21.11% and FKRG 1.11-17.36% at the various concentrations respectively. For both samples, the most significant inhibition approaching 20% occurred at the 125 µg/mL concentration. It is noteworthy however, that the inhibitory effect of these beverage samples were not significantly ($P > 0.05$) concentration dependent.



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B

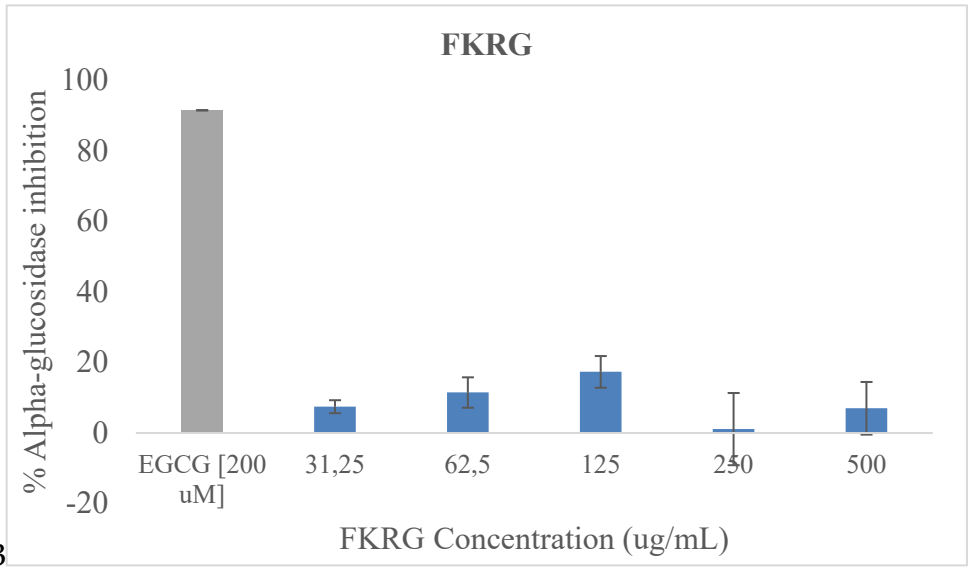


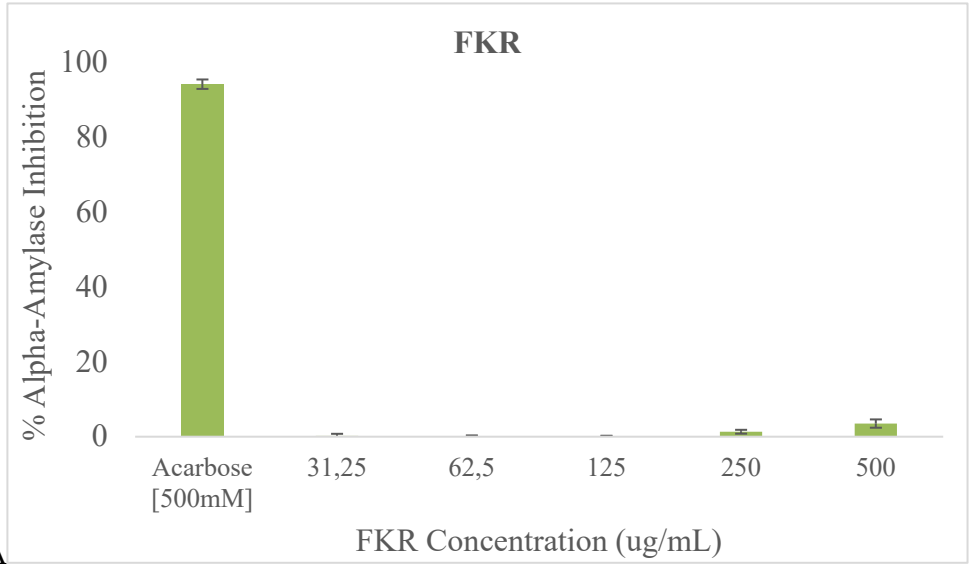
Figure 1. Alpha-glucosidase inhibition of A: Fermented Rice (FKR) beverage; B: Fermented Rice + Groundnut (FKRG) beverage.

ECGC [200 μM] was used as positive control. Values are means ± SD (n = 3); $P < 0.05$.

3.2. Alpha-amylase inhibition

Both FKR and FKRG exhibited moderate alpha-amylase inhibition activity at the tested concentrations, but very low inhibitory activity compared to acarbose (Figure 2). FKRG was found to have the higher alpha-amylase inhibition activity. For both samples, the most significant inhibition occurred at the 500 μg/mL concentration.

A



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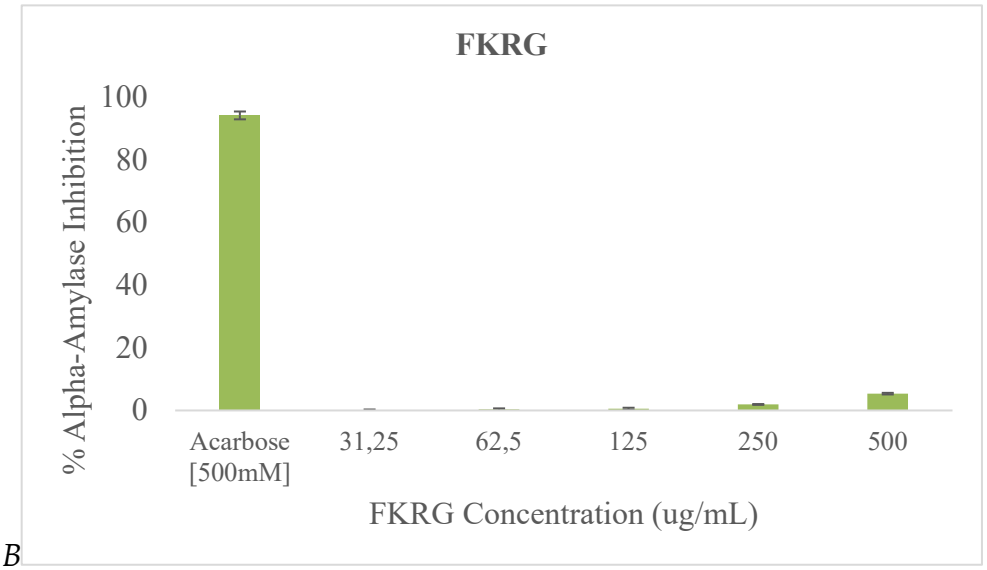


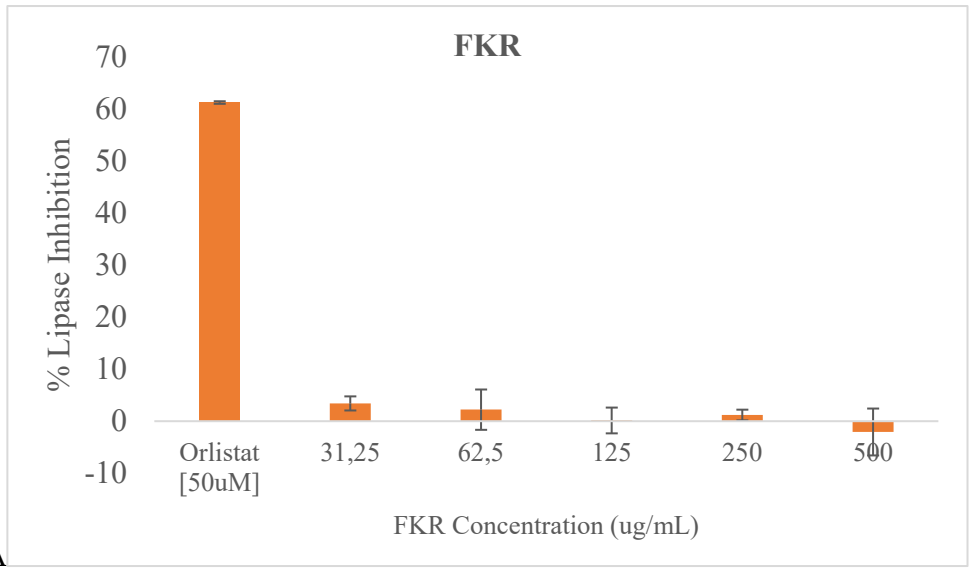
B

Figure 2. Alpha-amylase inhibition of A: Fermented Rice (FKR) beverage; B: Fermented Rice + Groundnut (FKRG) beverage. Acarbose [500 µM] was used as a positive control. Values are means ± SD (n = 3); $P < 0.05$.

3.3. Lipase inhibition

The potential pancreatic lipase inhibition activity of the two fermented rice beverages FKR and FKRG are illustrated in Figure 3.

No significant pancreatic lipase inhibitory activity was observed for sample FKR at the tested concentrations compared to Orlistat (a standard anti-obesity drug) used as control in this study. Although no significant inhibitory activity was observed in most of the tested concentrations for FKRG also, at 31.25 ug/mL there was an inhibitory effect of approximately 15%, which was not seen at the higher concentrations.



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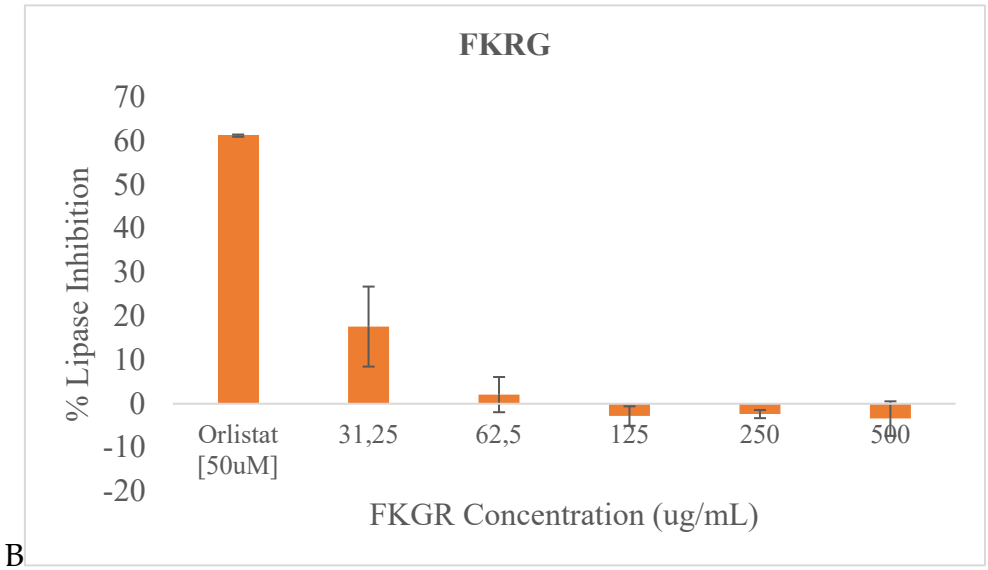

B

Figure 3. Pancreatic lipase inhibition of A: Fermented Rice (FKR) beverage; B: Fermented Rice + Groundnut (FKRG) beverage.Orlistat (50 μM) was used as a positive control. Values are means ± SD (n = 3); $p < 0.05$.

4. Discussion

Postprandial hyperglycaemia is a major contributing factor in the development of diabetes and represents an important therapeutic target. Anti-diabetic drugs that retard glucose absorption after a meal through the inhibition of carbohydrate hydrolysing enzymes are known to be effective in decreasing postprandial hyperglycaemia [26-28]. Therefore, retarding the absorption of glucose by inhibition of α-amylase and α-glucosidases (carbohydrate hydrolyzing enzymes), is a major therapeutic approach for decreasing postprandial hyperglycemia.

Alpha-Glucosidase is a membrane-bound enzyme located at the brush-border of epithelial cells in the small intestine, which catalyses the final stage of starch digestion by hydrolysing terminal glucose molecules from the non-reducing ends of oligosaccharides [29]. Thus, α-glucosidase inhibitors are frequently used as oral antidiabetic drugs in the early stages of type 2 diabetes to combat postprandial hyperglycaemia and obesity [30]. These inhibitors prevent postprandial hyperglycaemia by slowing down the digestion of carbohydrates and consequently the rate at which glucose can be absorbed into the general circulation. Acarbose and other α-glucosidase inhibitors act via a competitive inhibition mechanism and ideally bind to all four catalytic domains of the enzyme to effectively inhibit the hydrolysis of oligosaccharides [31].

Starch digestion is strongly associated with glycemia-related problems such as diabetes and metabolic syndrome including obesity and atherosclerosis. Pancreatic and salivary alpha-amylase and four small intestine mucosal alpha-glucosidase subunits are required to digest starch into glucose [32]. When given orally, acarbose inhibits alpha-glucosidase in the brush border of the small intestines and reduces the rate of digestion of complex carbohydrates. Inhibitors of alpha-glucosidase exhibit the same mechanism to retard glycation of proteins, thereby reducing glycated hemoglobin and glycation end products in collagen [33].

Alpha-amylase hydrolyses starch at inner alpha-1,4 glycosidic linkages to give linear and branched malto-oligosaccharides and disaccharides which then serve as substrates for intestinal alpha-glucosidases that hydrolyse the disaccharides to monosaccharides such as glucose [32,34]. Alpha-amylase is the limiting enzyme and therefore determines digestion and consequent rate of glucose release, thus alpha-amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha-amylase can convert starch to simple sugars [35].

Starch is hydrolyzed by pancreatic α-amylase into oligosaccharides, which is further hydrolyzed by α-glucosidase into glucose in the jejunum [36]. Therefore, glucose absorption could be delayed by

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impeding these two enzymes, thus suppressing the postprandial blood glucose levels. The moderate inhibition of α-glucosidase exhibited by the two fermented beverages FKR and FKRG indicates that both beverages have the potential to prevent postprandial hyperglycemia. The most significant inhibition (IC50) for both samples occurred at the 125 µg/mL, with inhibition approaching 20%.

The significant ($P < 0.05$) alpha-amylase inhibition exhibited by both samples, occurred at the 500 µg/mL testing concentration. This could be attributed to increased nutrient and polyphenolic content as a result of fermentation, as well as increase in antioxidant activity. This is so because during fermentation, plant cell walls are broken down liberating antioxidants, phytochemicals and nutrients present in foods are metabolized into simpler and unique health-beneficial components by the fermenting microorganisms [37]. In addition, several studies have reported that fermentation improves nutrient content and availability, polyphenolic content and enhances antioxidant content and capacity of functional foods through reduction or neutralization of tannins and phytates, resulting in greater bioavailability of nutrients, phytochemicals, vitamins and minerals amongst others [37-40].

The inhibitory activities of FRK and FRKG against α-amylase and α-glucosidase enzymes could be attributed to the additive polyphenolic contents resulting from the ingredients. According to Noviasari et al. [41], polyphenol and flavonoid content found in many foods have great capacity of inhibiting α-amylase and α-glucosidase. Moreover, the phenolic content of rice could be responsible for inhibition of the two enzymes, also legumes (peanuts in this case) and spices are known to be rich in protein, phytochemicals, dietary fibre, vitamins, and other micronutrients beneficial for human health [42-43]. These results agree with Ramakrishna et al. [44] who also reported low to moderate alpha-glucosidase inhibition for fermented barley flour extracts. When enzymes involved in starch degradation are inhibited, the release of glucose is slowed down and its level in the blood is reduced [45].

Dietary lipid digestion occurs through the hydrolysis of triacylglycerols within the intestinal lumen to monoacyl glycerols by pancreatic lipase. This inhibition is one of the most promising methods for combating obesity and hypertriglyceridemia in type 2 diabetes [46]. Although the inhibitory action against pancreatic lipase was very low for FKR and FKRG compared to orlistat the standard drug, at 31.25 ug/mL, there was a significant ($P < 0.05$) inhibitory effect of approximately 15%, by FKRG which was absent at higher concentrations. Insulin resistance has been associated with dyslipidemia (hypertriglyceridemia and low HDL-cholesterol) and could lead to increased synthesis and secretion of very low-density lipoprotein, resulting in increased plasma triglyceride levels and decreased plasma HDL-cholesterol levels.

According to Ignat et al. [42], non-alcoholic cereal-based beverages are healthy drinks that impact positively on human health and their consumption improves liver function, gut microbiota and reduces the incidence of nosocomial infections. Although, fermented foods and beverages may not replace medical treatment, they could serve as adjuvants or alternative therapies in treating and managing the condition through reducing oxidative stress, glycemic control, recovery or maintenance of body weight [43,47].

Limitations of the study

- The use of spontaneous fermentation (native flora) implies that different populations of microorganisms are involved.
- Use of the freeze-dried filterate from the rice beverage could have been responsible for the low/moderate inhibitions observed, since the beverage is supposed to be consumed as a slurry.
- Also, no study on the fermenting organisms of the beverages were performed.

5. Conclusions

The present results reveal the potential of fermented rice beverage to moderately inhibit carbohydrate metabolizing enzymes (α-amylase, α-glucosidase) and pancreatic lipase *in vitro* and

could modulate postprandial hyperglycemia in type 2 diabetes. Again, better inhibitory action of the fermented beverages against α-amylase, α-glucosidase and pancreatic lipase may be possible *in vivo*, which thus calls for further studies.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, G.A.O.; Methodology, G.A.O.; O.T.T.; C.O-Y.; J.N.; Formal Analysis, G.A.O.; and O.T.T.; Data Curation, G.A.O.; Writing–Original Draft Preparation, G.A.O.; and O.T.T.; Writing–Review & Editing, G.A.O.; OT.T.; C.O-Y.; J.N.; O.O.; and A.J.A.; Supervision, G.A.O.; Funding Acquisition, G.A.O.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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