The Bioavailability of Nutrients, Antioxidants, and Short-Chain Fatty Acids in vitro Fermentation of Hydrolyzed Canned Red Kidney Beans (Phaseolus vulgaris L.) in a Simulated Gastrointestinal Tract System

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RESEARCH

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ABSTRACT

The bioavailability of nutrients could be improved using in vitro fermentation of hydrolyzed RKB because of the rich carbohydrate contents. Therefore, we hypothesized that in vitro fermentation of enzymatic hydrolyzed RKB will enhance the production of short-chain fatty acids, antioxidant capacity, and minerals in the gastrointestinal tract (GIT). The study's objective was to determine the impact of in vitro fermentation on the bioavailability of nutrients, antioxidants, and short-chain fatty acids in a simulated GIT system. The simulation of the oral, gastric, and intestinal digestion phases was conducted in a bioreactor. The pre-sterilized media prepared was inoculated with pig fecal slurry. The bioreactors were kept in an anaerobic condition at 37°C, agitated at 70 rpm for 24 h. Aliquots were collected, centrifuged, and analyzed for short-chain fatty acids, antioxidants, and minerals. The presence of SCFA (acetic, propionic, and butyric acid) was detected after 24 h in vitro fermentation by Pig fecal colonic microflora with propionic acid having the highest concentration of 47 mg/L using a Gas Chromatography. The total phenolic and total flavonoid contents after in vitro fermentation were 299 mgGAE/100 g DW RKB and 313 mgCE /100 g DW RKB, respectively, without enzymatic hydrolysis. With enzymatic hydrolysis, 265 mgGAE/100 g DW RKB and 215 mg CE/100 g DW RKB, respectively. The P, Mg, Ca, Fe, Zn and Cu were 13997, 801, 634, 75, 18, and 10 µg/kg. This study shows that hydrolyzed RKB has health benefits and can be used in promoting health.

Key words: Antioxidants, short-chain fatty acid, DPPH, FRAP.

Abbreviations

AA	-	Acetic Acid
Ca	-	Calcium
$CaCl_2$	-	Calcium Clolride
Cu	-	Copper

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DPPH	-	2, 2-Diphenyl-2-Picrylhydrazyl
Fe	-	Iron
FRAP	-	Ferric Reducing Antioxidant Potential
FTU	-	Concentration of Enzyme
GAE	-	gallic acid equivalents
GI	-	Gastrointestinal
GIT	-	Gastrointestinal Tract
HCL	-	Hydrochloric Acid
ICP	-	Inductively Coupled Plasma
K_2HPO_4	-	Dipotassium Phosphate
$MgSO_4$	-	Magnesium Sulfate
NaCl	-	Sodium Cloride
$NaHCO_3$	-	Sodium Bicarbonate
PA	-	Propionic Acid
P	-	Phosphorous
RKB	-	Red Kidney Beans
SCFA	-	Short-Chain Fatty Acids
TFC	-	Total Flavonoidfs Content
TPC	-	Total Phenolic Content
Zn	-	Zinc

INTRODUCTION

In vitro, digestion simulates the lower part of the gastrointestinal tract (GIT); hence, it is used to study how foods and bioactive compounds are digested [1]. The resident bacteria and other microorganisms in the gastrointestinal (GI) tract are called microbiota. The human colon's microbiota is composed of different bacteria that ferment dietary carbohydrates such as dietary fiber, which are not digested in the small intestine, leading to the formation of short-chain fatty acids (SCFA). These SCFA act as energy sources for microbial growth and have physiologic effects on the host [2, 3]. According to Bergman [4], SCFA provides about 10% daily caloric requirements of humans. The daily requirements depend on the amount of daily fiber consumed, the proportion of fermentable fiber, and the composition of the GI tract. The SCFA reduces the pH, thereby stimulating the development of beneficial bacteria such as Lactobacillus and Bifidobacterium [5, 6]. The SCFAs also enhances the absorption of minerals, and promotes the excretion of bile acid [7], and suppresses the development of toxic metabolite producing bacteria [8].

Red kidney beans (RKB), Phaseolus vulgaris L contain soluble and insoluble dietary fibers, hence know to be one of the best sources dietary fibers. They are an excellent and inexpensive source of protein, carbohydrate, minerals, and vitamin [9, 10, 11. 12, 13]. In addition to being rich sources of several macro and micronutrients, dry beans contain oligosaccharides and essential bioactive compounds such as polyphenols that may benefit human health. The bioavailability of minerals in red kidney beans is low because of the presence of anti-nutritional factors such as phytic acids, which form insoluble complexes during digestion [14]. Enzymatic hydrolysis has been found to improve and increase antioxidants' release and beans' digestibility [15].

The gut microbiome obtained from the fecal matter of pigs in an animal study was used as an inoculum to hydrolyze the non-digestible fraction of the carbohydrate (dietary fibers) from the RKB [16]. The process then converted the carbohydrate (dietary fibers) into simple sugars during the anaerobic fermentation in the GIT [17]. As a result, the short-chain fatty acids (propionic acids, acetic acids, butyric acids) produced are metabolites derived from the carbohydrates' fermentation. These are saturated, aliphatic organic acids, which consist of one to six carbons. Furthermore, the high level of dietary antioxidants (polyphenols and flavonoids) and minerals (P, Ca, Fe, Zn, Cu) in the RKB play an essential role in protecting the GIT tract from oxidative damage and delays the development of the stomach, colon, and rectal cancer [18]. Therefore, in the present study, the in vitro intestinal fermentation experiment with pig fecal matter to simulate the human colon was conducted to elucidate the effect of hydrolyzed RKB on the production of SCFA, nutrients, and antioxidants.

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MATERIALS AND METHODS

Simulation digestion studies

A simulation study of the digestive system was conducted according to the modified method described by Nderitu et al. [19]. This simulation digestion model consists of a 3-step procedure that simulates the digestion in the oral (mouth), stomach, and intestine as shown in Figure 1. The canned RKB samples were randomly selected from each treatment group (0 FTU brine, 0 FTU buffer, 200 FTU brine, and 200 FTU buffer) solutions. The randomly selected canned RKB samples (30 g) were put into a flask and homogenized in 30 mL saline using a tissue tearor (Model S85-370, Biospec Products, Inc.). Salivary fluid: 0.15 M NaCl and 3 mM Urea solution (24 mL) was added, followed by α-amylase (40 µL) and incubated for at 37°C in a reciprocal shaking bath (Model 2872, Thermoscientific, Marietta, Ohio) for 2 minutes for oral digestion. An aliquot was removed to analyze TPC, TFC, FRAP and kept at -20°C. This was followed by 2 h gastric digestion where the pH of the RKB samples from the oral digestion was adjusted to 2 using pepsin (1.875 mL) and 1 M HCL and incubated at 37°C in a reciprocal shaking water bath (Model 2872, Thermoscientific, Marietta, Ohio) at 70 rpm.

After the gastric digestion phase, an aliquot was removed and stored in centrifuge tubes. The RKB samples' pH was adjusted to 7 using 1 M NaHCO3 solution and 3 mL of pancreatin-bile salt mixture for intestinal digestion and incubated for 2 h. After incubation, the enzymes were deactivated by heating the samples at 70°C for 20 minutes using a reciprocal shaking bath (Model 2872, Thermoscientific, Marietta, Ohio). The RKB samples were kept immediately in the refrigerator to cool overnight. The RKB samples were centrifuged at 3000g at 25° C for 15 min using Sorvall legend XTR Centrifuge (Model XTR, Thermofisher Scientific, Ridgefield, CT). The residue obtained from the centrifugation was stored immediately at -20° C and subsequently freeze-dried using virtis genesis pilot lyophilizer (Model# 25L Genesis SQ Super XL-70, Stone Ridge, New York) for in vitro fermentation.

Preparation of Media

The media was prepared according to the method described by Mandalari et al. [20] with modifications. Four bioreactors (300 mL) were filled with 135 mL of pre-sterilized basal growth medium that consisted of 2 g/liter peptone water, 2 g/liter yeast extract, 0.1 g/liter NaCl, 0.04 g/liter K2HPO4, 0.04 g/liter KH2PO4, 0.01 g/liter MgSO4.7H2O, 0.01 g/liter CaCl2.6H2O, 2g/liter NaHCO3, 2 mL Tween 80, 0.02 g/liter hemin, 10 µL vitamin K1, 0.5 g/liter cysteine HCL, and 0.5 g/liter bile salts, pH 7.2.

Inoculum Preparation

The inoculum (Pig feces) was received from Auburn University and stored immediately in a -85°C deep freezer (Model VX380, Jouan SA, Herblain, France). The fecal matter was thawed to 37°C using the Incu Shaker Mini Magic Clamp Platform (Model 115 VAC, Benchmark Scientific, Edison, New Jersey). A day before the anaerobic fermentation, the fecal slurry was prepared using 10 g of the fecal material of pig mixed with 100 mL 0.1 M phosphate-buffered saline (pH 7.26). This was prepared in a stomacher bag and homogenized using a bag mixer machine (Model 400 W, Interscience laboratories Inc., Woburn, MA).

In vitro Fermentation Process

The pre-sterilized growth medium prepared was inoculated with 15 mL of the fecal slurry. The ground freeze-dried RKB samples residue from the simulation digestion was added (1 g) to each of the bioreactors. The pH of the media was checked and paraffin films were used to cover each bottle. Nitrogen gas was infused in each of the bioreactors to purge the dissolved oxygen from the solution. The bioreactors were then placed inside the anaerobic glass jar and a vacuum pump (Model 2546B-01, Welch, Monroe, Louisiana) was used to evacuate any oxygen from the system. Nitrogen gas was pumped in again inside the anaerobic fermentation jar to remove any oxygen from the vessel. The anaerobic jar was placed in a heated shaking water bath (Model 2874, Dubnoff Metabolic Shaking Incubator, Boston, MA) at 37°C and 70 rpm speed,

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and incubated for 24 h. The incubation was stopped 24 h and the samples were allowed to equilibrate to room temperature. The pH was checked using a pH meter (Thermoscientific Orion 3 star benchtop pH meter, Beverly, MA). After that, the samples were centrifuged using a Sorvall refrigerated superspeed centrifuge (Model ST8, Thermoscientific, Newport, CT) at 15,000 x g for 5 min. The aliquot was recovered and stored prior to the short-chain fatty acids, antioxidants, and ICP analysis.

Determination of Short-Chain Fatty Acid after Anaerobic Fermentation using Gas Chromatography (GC)

Short-chain fatty acid (SCFA) analysis was carried out according to the modified method of Mandalari et al. [20]. The aliquot collected after anaerobic fermentation was used to determine the SCFA present using the GC (Varian 3800, Agilent, Santa Clara, California). The SCFA was separated using a DB-FFAP column for SCFA with a flame ionization detector (FID) detector. A syringe was used to collect 1µL of the samples which were injected. The temperature ranged from 100-240°C with a flow rate of 2.0 mL/min. The quantification of the SCFA was estimated based on the retention time peak and areas of the acetic, propionic, and butyric acid curves at concentrations between 0 and 1000 ppm. The results were expressed in mg/L.

Determination of Total Phenolic Content (TPC)

The TPC of the aliquots were used to determine the phytochemical and antioxidant activities. The method described by Gajula et al. [21] using the Folin-Ciocalteu reagent was used. Gallic acid standard concentrations of 0.02, 0.04, 0.06, 0.08, and 0.10 (mg/mL) were prepared. The sample and standard solution (12.5 µL) were pipetted into 96 well plate cells. Folin- Ciocalteus solution (12.5 µL) was added to each cell and incubated for 5 min, followed by adding 125 µL of 7% sodium carbonate and, subsequently, 50 µL of deionized water. The plate was incubated in the dark at room temperature for 90 min for a color reaction. The mixture's absorbance was measured at 750 nm using a microplate spectrophotometer (Spectra Max 150, Molecular Devices, Sunnyvale, California). The results were expressed as mg of gallic acid equivalents (GAE) per g of the dry weight of red kidney beans (mg GAE/100 g DW).

Determination of Total Flavonoids Content (TFC)

The TFC of the aliquots were used to determine the phytochemical and antioxidant activities. The method described by Gajula et al. [21] was used. The standard solutions of catechins at concentrations of 0.02, 0.04, 0.06, 0.08, and 0.10 (mg/mL) were prepared. Briefly, sample or standard (25 µL) solutions were added in the 96 well plate cells, and 7.5 µL of sodium nitrite was added and incubated for 5 min. This was followed by the addition and incubation of 15 µL aluminum chloride added for 5 min, and then, 50 µL sodium hydroxide was also added and left for 5 min. 40 µL of deionized water was subsequently added, and the absorbance was read at 510 nm using a microplate spectrophotometer (Spectra Max 150, Molecular Devices, Sunnyvale, California). The TFC was determined from the standard curve based on catechin acid. Results were expressed as mg catechins acid equivalents per g of the dry weight of RKB (mg CAE/100 g DW).

Determination of FRAP

The ferric reducing antioxidant potential (FRAP) of the aliquots was determined using the method described by Chen et al. [22]. A standard solution of FeSO4.7H20 was prepared at the following concentrations of 0.1, 0.25, 0.50, 0.75, and 1.0 (mM). The standard and sample (10µL) were pipetted into 96 well plate cells, and 280 µL of the FRAP reagent (Acetate buffer, TPTZ, FeCl2) solutions were added and followed by the addition of 10 µL of water (optional). The absorbance was read at 593 nm for 4 min durations at 1 min interval using a microplate spectrophotometer (Spectra Max 150, Molecular Devices, Sunnyvale, California).

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Determination of 2, 2-Diphenyl-2-Picrylhydrazyl (DPPH) scavenging capacity

The DPPH radical scavenging assay of the aliquots was measured according to the modified method of Chen et al. [22]. The DPPH stock solutions were prepared daily before measuring with the microplate spectrophotometer (Spectra Max 150, Molecular Devices, Sunnyvale, California) using 0.001 g DPPH and 50 mL 80% ethanol. The samples were diluted with water at following concentrations: 0.2, 0.4, 0.6, 0.8, and 1.0 (mg/mL). Briefly, 40µL of the standard or the sample extract at various concentrations were placed in a 96-well microplate. Then, 200 µL of freshly prepared DPPH stock solution was added. The absorbance was measured at 517 nm against a blank at 30 min intervals for 90 min using a microplate spectrophotometer (Spectra Max 150, Molecular Devices, Sunnyvale, California). Each sample was measured in triplicate (n=3). The DPPH scavenging effect was calculated using equation 1:

$$Inhibition(\%) = \left(\frac{Acontrol - A\,sample}{A\,control}\right) X\ 100 \quad (1)$$

Where, Acontrol is the absorbance of the blank and Asample = absorbance of the sample extract.

Instrumental Analysis of the Mineral Content

The aliquots collected after in vitro fermentation of RKB were analyzed for mineral content using the ICP-OES (Model Optima 2100DV, PerkinElmer Inc., Wellesley, MA). After creating the methods, the elements Mg, Ca, Cu, Zn, P, and Fe were selected for analysis. The system went through a verification check and the standards and the blank were subsequently analyzed to ensure the system functionality. The samples were then run data collected.

Statistical analysis

The data obtained from the experiment were analyzed using Analysis of Variance (ANOVA) using the SAS 9.0 statistical programs. Duncan's group mean comparison test was used for the mean comparisons of significant treatments. All experiments were carried out in triplicates, and statistical tests were performed at a 5% level of significance.

RESULTS AND DISCUSSIONS

Short-chain fatty acid

The fermentation of hydrolyzed RKB led to the production of SCFA's. This study looked at the production of SCFA's after the digestion of hydrolyzed RKB extracts. After 24 hours of in vitro fermentation, a decrease in the pH was observed. Fermentation in the lower gut is associated with a reduction in pH, which aids in the increased beneficial microorganisms such as Lactobacillus [23]. Change in pH is an indicator to ascertain if fermentation occurred after 24 h. The results showed a decrease in pH after 24 h of fermentation at 37°C, with the brine solution at 0 FTU showing the lowest pH of 6.39. The reduction in pH could be associated with the production of SCFA. Thus, the validation of the presence of SCFA was determined using the aliquot collected after the in vitro fermentation. Hernandez-Salazar et al. [23] on the in vitro fermentability of indigestible fraction of cooked black beans showed a reduction in pH of 6.35 after 24 h, which is also similar to the pH recorded in this study.

The ANOVA for the propionic acid (PA) after in vitro fermentation shows the level of enzymatic concentration (FTU) had a significant difference ($p<0.05$) in the production of PA after in-vitro fermentation of RKB. Since a significant difference was observed for the enzyme concentration on PA concentration, a mean separation test was conducted using Duncan's mean separation test, which showed there was a difference between 0 and 200 FTU on the production of PA. The propionic acid produced after the fermentation of hydrolyzed RKB with no enzyme (0 FTU) was 18±3 mg/L and 47± mg/L at 200 FTU. The hydrolyzed RKB extracts showed the highest production of SCFA's after 24 h in vitro fermentation. There were traces of butyric acid on the RKB hydrolyzed at 0 and 200 FTU. According to Hernandez-Salazar et al. [23], the indigestible fraction of chickpea and lentil did not produce propionic or butyric acids during the

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12 h fermentation; however, after 24 h, the production of butyric acid was observed for black beans, lentils, and chickpeas. In their study, black beans produced the highest acetic acid level, and lentils yielded the highest amount of propionic acid. In this study, propionic acid was the highest level of short-chain acid detected after the in vitro fermentation. The differences in the SCFA levels could be attributed to the type of legume used in the study and the treatments. The type of undigested fraction in the hydrolyzed RKB determines the kind of SCFA produced after in vitro fermentation. A study conducted by Nagata et al. [24] showed a reduction of butyrate concentration after in vitro fermentation of adzuki beans extract and a combination of inulin.

Polyphenols and their metabolites can modify the microbiota [25, 26, 27]. This can, in effect, affect the level of the intestinal fermentation metabolites [28, 29]. A study conducted by Nagata et al. [24] stated that the polyphenols present in the adzuki beans extract could have affected the microbiota's composition, thus reducing the production of butyrate. This is also similar to this study because traces of butyric acid were observed after the in vitro fermentation, which shows that the polyphenols present in the RKB could have inhibited butyric acid production [24]. According to den Besten et al. [17], bacteria that belong to phylum Bacteriodetes are known to produce mainly acetate with propionate, while bacteria that belong to phylum Firmicutes produce butyrate as their primary end product. From the results obtained from this study, it could be that the Bacteriodetes were the most prevalent after the fermentation process because of the production of propionic and acetic acid.

Even though no significant difference ($p > 0.05$) was observed for the RKB extracts hydrolyzed at 0 and 200 FTU on the acetic acid (AA) production, the highest was observed at 200 FTU (32 ±1 mg/L), and 22 ±2 for the AA produced with no enzyme (0 FTU) after in vitro fermentation of hydrolyzed RKB. Work conducted by Hernandez-Salazar et al. [23] showed the highest AA production in black beans after 24 h of fermentation to be 26.3±0.2 mg/L, while Mandalari et al. [30] reported 15.05 ±2.43 mM for almond seeds as their experimental control and 50.77 ±3.91 mM for prebiotic (fructooligosaccharide) after 24 h.

Figure 2 showed that the 200 FTU concentration produced the highest PA (47 ±3.3 mg/L) than at the 0 FTU (18 ±3.3 mg/L). Mandalari et al. [30] reported propionic concentration for almond after 24 h to be 3.90 ±0.70 mM, and 11.11±2.27 mM for the fructooligosaccharide after 24 h in vitro fermentation. Figure 3 shows the acetic acid produced after the fermentation of hydrolyzed RKB extracts.

Acetate was the highest SCFA detected in vitro digested cooked cowpea and black beans in a study conducted by Teixeira-Guedes et al. [31] and the acetate measured was 42 mM after 48 h fermentation. The propionate detected in the same study was about 5.9 mM after 48 h fermentation, and a low concentration of butyrate (1.61 mM) was measured. The study shows that cooked cowpea and black bean are be suitable substrates to support the growth of beneficial microorganisms such as Bifidobacterium and Lactobacillus, hence, aligned to our finding of this study with regards to the production of SCFA. Dietary carbohydrates that were not digested in the small intestine is transported to the colon are fermented by the beneficial microorganisms, hence leading to the formation of SCFA. These SCFA help decrease the risk of developing gastrointestinal disorders, cancer, and cardiovascular diseases [32]. The rate of digestion [33] and the food type [34] are factors that affect the digestibility of carbohydrates in the small intestine. The development of functional foods in the form of prebiotics will help to stimulate the growth of beneficial bacteria such as Bifidobacteria and Lactobacillus when consumed. Nutrition thus plays a vital role in dealing with the problem associated to gastrointestinal health. For example, low consumption of dietary carbohydrates was reported to decrease diseases, such as diabetes and cancer [32].

The undigested part of plant foods (source of fiber) are the insoluble fibers that are beneficial to the

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gastrointestinal health. Dietary carbohydrate provides energy to the body to support some special functions in the body. When the body utilizes its energy, it stores the remaining carbohydrate in the body as glycogen. There is a strong relationship between the consumption of dietary carbohydrate and metabolism fat and energy stored [35]. Thus, consumption of excess carbohydrates can also lead to obesity, because this inhibit the body's potential to utilize the proteins as the source of energy. The results indicated that the fibers RKB contribute towards eliminating solid wastes by acting as a laxative, hence improves satiety, which will helps to reduce the amount of food consumed.

Nutrients

After the in vitro digestion of hydrolyzed RKB extracts, the mineral concentrations were determined using Inductively Coupled Plasma (ICP). Table 1 shows the mineral profile of in vitro fermented hydrolyzed canned RKB. The graph shows that phosphorus had the highest concentration of 13997 ug/kg. The work conducted by Tasie et al. [36] on the mineral profile of raw RKB showed the raw RKB with a concentration of 3184 mg/kg. The increase could be attributed to dry matter loss during the in vitro fermentation and microorganisms' degrading carbohydrates and protein present in the RKB [37]. This trend of the increase was also observed in iron and copper, which increased after in-vitro fermentation compared to the raw RKB. The iron and copper concentration of the raw RKB was 56 mg/kg and 4.8 mg/kg, as documented by Tasie et al.[36]. The minerals also increased after the in vitro fermentation resulting from the breakdown of phytates that complex with these minerals making them less bioavailable [38].

According to Nkhata et al. [39], the low pH observed after fermentation could have also increased the absorption of iron due to the conversion from ferrous iron that is less absorbable to the ferric iron, which is more readily absorbed. As seen in the results, the iron content increased from 56 mg/kg (raw) to 75 mg/kg after in vitro fermentation. These findings agreed with Zhang et al. [40], who determined the bioavailability of minerals in different pulses using in vitro gastrointestinal fermentation. Their study detected Fe, P, Ca, Zn in small quantities during intestinal digestion but was released in the colon in large amounts. Large amounts of Ca, Mg and P were also detected in the human colon [41, 42].

The enzyme, Maxamyl phytase, used to hydrolyze the RKB, and the endogenous enzymes could have contributed to the degradation of phytic acid, making nutrients more bioavailable. The microorganisms from the Pig fecal matter could have also facilitated the fiber's degradation in the hydrolyzed RKB, making nutrients available. Magnesium, Ca and Zn also were available after the in-vitro fermentation, although the concentrations were higher in the raw RKB. Overall, the availability of minerals shows that even after the in vitro fermentation of fibers by the microorganisms, there were still minerals present.

Mean values with different letters within the same column show significant differences ($p \leq 0.05$) based on Duncan's multiple comparison test.

Antioxidants

Although the total phenolic content (TPC) was not significantly different ($p > 0.05$), the TPC determined after in vitro fermentation at 0, and 200 FTU were 299 mgGAE/100 g and 265 mgGAE/100 g, respectively (Figure 4). The presence of TPC after in vitro fermentation of hydrolyzed canned digested RKB shows that the TPC was stable during the in vitro fermentation process and was then able to be released. Hernandez-Salazar et al. [23] stated that pulses contain complex carbohydrates that digest slowly but still are sources of polyphenols.

Zhang et al. [43] utilized in vitro simulated digestion to determine polyphenol and antioxidant contents of quinoa and djulis sprouts after gastric digestion, and showed the TPC increased dramatically, after intestinal digestion. Tagliazucchi et al. [44] also showed phenolic compounds are released during the digestion phase. Many other studies have found phenolic compounds to be released from plant materials during the digestion process because of the digestive enzymes and the pH as they are

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absorbed in the GIT. Hence, this may be the cause of TPC increased at the gastric and intestinal phases of digestion [45]. A study conducted by Zhang et al. [43] also reported a high TFC content of quinoa after the gastric and intestinal phases of digestion, which were higher than the TFC obtained after the oral digestion phase.

During the colonic fermentation of indigestible fractions in pulses, polyphenols may have been liberated and, thus, exert their antioxidant capacity in the colon [23]. According to Saura-Calixto et al. [47], condensed tannins have low bioaccessibility in the small intestine but are available in the large intestine. In this study, antioxidants were detected after in vitro fermentation, showing that antioxidants were available.

After the in vitro fermentation of the hydrolyzed canned digested RKB, the enzyme concentration significantly ($p<0.01$) impacted the total flavonoid content (TFC). The TFC was more released at 0 FTU (313 CAE mg/100 g DW) than at 200 FTU (215 CAE mg/100 g DW) RKB. Gan et al. [48] reported a TFC content of kidney bean seed coats as 3513 mg CE/100 g. Some of the TPC and TFC may have leached in water because of the fermentation process. During in vitro fermentation, the microorganisms present could have caused the breakdown of linkages of TFC, making them readily available [49].

A significant difference was observed ($p<0.05$) for the FTU in the availability of FRAP after the in vitro fermentation of hydrolyzed, canned, digested RKB. Figure 5 describes the FRAP results of RKB after in vitro fermentation based on the Duncan mean analysis. The FRAP value at 0 FTU had a higher reducing power than the RKB hydrolyzed at 200 FTU. The red color of RKB also seems to have influenced the antioxidant activity. According to Adetuyi and Ibrahim [50], fermentation improved the DPPH and okra seeds' FRAP activity and Haile at al. [51] also reported that the fermentation of green coffee beans with W. anomalus improved the FRAP and DPPH activity.

The enzyme concentration did not significantly impact ($p>0.05$) the DPPH content after the in vitro fermentation of hydrolyzed canned digested RKB. Figure 6 shows the effect of enzyme concentration on the % DPPH inhibition of in vitro fermented RKB. Although the DPPH content was low and not significant, there was still some on the RKB extracts even after in vitro fermentation, which shows that they could scavenge free radicals.

CONCLUSION

This study investigated the bioavailability of nutrients, SCFAs, and antioxidants during the in vitro fermentation of hydrolyzed canned RKB in a simulated gastrointestinal tract. There was a significant production of short-chain fatty acids- propionic acid, acetic acid, and traces of butyric acid. There was a significant increase in the antioxidant capacity- total phenolic and flavonoid contents, DPPH, and FRAP. There was a significant increase in phosphorus release, magnesium, calcium, iron, zinc, and copper. This study shows that the enzymatic hydrolysis process significantly enhanced the bioavailability of nutrients, SCFAs, antioxidant potential in RKB and may have substantial health benefits.

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REFERENCES

1. Hur, S. J et al. (2011). In vitro human digestion models for food applications. Food Chemistry, 125, 1-12.

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2. Holscher, H. D. (2017). Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes, 8, 172-184.

3. Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell, 165, 1332-45.

4. Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews, 70, 567–90.

5. Topping, D. L., Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews, 81, 1031–64.

6. Bornet FR, & Brouns F. (2002). Immune-stimulating and gut health-promoting properties of short-chain fructooligosaccharides. Nutrition Reviews, 60, 326–334.

7. Alexander, C., Swanson, K.S., Fahey Jr., G. C., & Garleb, K. A. (2019). Physiologic Importance of Short-Chain Fatty Acids from Nondigestible Carbohydrate Fermentation. Advances in Nutrition, 10, 576-589.

8. Gibson, G. R, McCartney, A. L, & Rastall, R. A. (2005). Prebiotics and resistance to gastrointestinal infections. British Journal of Nutrition, 93, S31–S34.

9. Anderson, J. W., Smith, B. M., & Washnock, C. S. (1999). Cardiovascular and renal benefits of dry bean and soybean intake. Am J Clin Nutr, 70(3), 465S-474S.

10. Osorio-Diaz, O., Bello-Perez, L. A., Agama-Acevedo, E., Vargas-Torres, A., Tovar, J., & Paredes-Lopez, O. (2002). In vitro digestibility and resistant starch content of some industrialized commercial beans (Phaseolus vulgaris L.). Food Chemistry, 78, 333-337.

11. Vargas-Torres, A., Osorio-Diaz, P., Islas-Hernandez, J. J., Tovar, J., Paredes-Lopez, O., & Bello-Perez, L. A. (2004). Starch digestibility of five cooked black bean (Phaseolus vulgaris L.) varieties. J Food Compos Anal., 17, 605–612.

12. Pisonero-Vaquero, S., Martinez-Ferreras, A., Garcia-Mediavilla, M. V., Martinez-Florez, S., Fernandez, A., Benet, M., Olcoz, J. L., Jover, R., Gonzalez-Gallego, J., & Sanchez-Campos, S. (2015). Quercetin ameliorates dysregulation of lipid metabolism genes via the PI3K/AKT pathway in a diet-induced mouse model of nonalcoholic fatty liver disease. Mol. Nutr. Food Res., 59, 879–893.

13. Rehinan, Z. U., Rashid, M., & Shah, W. H. (2004). Insoluble dietary fiber components of food legumes as affected by soaking and cooking processes. Food Chem, 85, 245–249.

14. Alegr´ıa-Toran, A., Barber ´ a-S ´ aez, R., & Cilla-Tatay, A. (2015). Bioavailability of minerals in ´ foods. In M. G. de la & S. Guardia (Eds.), Handbook of mineral elements in food (pp. 41–63). Hoboken: Wiley Blackwell.

15. de Souza Rocha, T., Hernandez, L. M. R., Chang, Y. K. & de Mejía, E. G.(2014). Impact of germination and enzymatic hydrolysis of cowpea bean (Vigna unguiculata) on the generation of peptides capable of inhibiting dipeptidyl peptidase IV. Food Research International, 64, 799–809.

16. Blackwood, A. D., Salter, J., Dettmar, P. W., & Chaplin, M. F. (2000). Dietary fiber, physicochemical properties, and their relationship to health. Journal of the Royal Society for the Promotion of Health, 120, 242-247.

17. den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. Journal of Lipid Research, 54, 2325–2340.

18. Halliwell, B., Zhao, K., & Whiteman, M. (2000). The gastrointestinal tract: a major site of antioxidant action? Free Radical Research, 33, 819-830.

19. Nderitu, A. M., Dykes, L., Awika, J. M., Minnaar, A., & Duodu, K. G. (2013). Phenolic composition and inhibitory effect against oxidative DNA damage of cooked cowpeas as affected by simulated in vitro gastrointestinal digestion. Food Chemistry, 141, 1763-1771.

20. Mandalari, G., Tomaino, A., Arcoraci, T., Martorana, M., Turco, V. L., Cacciola, F., Rich, G. T., & Wickham, M. S. J. (2010). Characterization of polyphenols, lipids, and dietary fiber from almond skins (Amygdalus communis L.). Journal of Food Composition and Analysis, 30, 1-9.

21. Gajula, D., Verghese, M., Boateng, J., Walker, L. T., Shackelford, L., Mentreddy, S. R., & Cedric, S. (2009). Determination of total phenolics, flavonoids, and

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antioxidant and chemopreventive potential of basil (Ocimum basilicum L. and Ocimum tenuiflorum L.). International Journal of Cancer Research, 5(4), 130-143.

22. Chen, Y., Zhang, H., Wang, Y., Nie, S., Li, C., & Xie, M. (2014). Acetylation and carboxymethylation of the polysaccharide from Ganoderma atrum and their antioxidant and immunomodulating activities. Food Chemistry, 156, 279-288.

23. Hernandez-Salazar, M., Osorio-Diaz, P., Loarca-Pina, G., Reynoso-Camacho, R., Tovar, J., & Bello-Perez, L. A. (2010). In vitro fermentability and antioxidant capacity of the indigestible fraction of cooked black beans (Phaseolus vulgaris L.) lentils (Lens culinaris L.) and chickpeas (Cicer arietinum L.). Journal of Science Food Agriculture, 90, 1417-1422.

24. Nagata, R., Echizen, M., Yamaguchi, Y., Han, K., Shimada, K., Ohba, K., Kitano-Okada, T., Nagura, T., Uchino H., & Fukushima, M. (2018) Effect of a combination of inulin and polyphenol-containing adzuki bean extract on intestinal fermentation in-vitro and in-vivo, Bioscience, Biotechnology, and Biochemistry, 82(3), 489-496.

25. Selma, M. V., Espín, J. C., & Tomás-Barberán*, F. A. (2009). Interaction between Phenolics and Gut Microbiota: Role in Human Health. Journal of Agricultural and Food Chemistry, 57(15), 6485–6501.

26. Zduńczyk Z, Juśkiewicz J, Estrella I. (2006). Cecal parameters of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in a combination of rats fed diets containing grapefruit polyphenols and inulin as single supplements or in a combination. Nutrition, 22, 898–904.

27. van Duynhoven, J., Vaughan, E. E., van Dorsten, F., Gomez-Roldan, V., de Vos, R., Vervoort, J., van der Hooft, J. J. J., Roger, L., Draijer, R., & Jacobs, D. M. (2013). Interactions of black tea polyphenols with human gut microbiota: implications for gut and cardiovascular health. The American Journal of Clinical Nutrition, 98(6), 1631S–1641S.

28. Sonoyama, K., Ogasawara, T., Goto, H., et al. (2010). Comparison of gut microbiota and allergic reactions in BALB/c mice fed different cultivars of rice. Journal of Nutrition, 103, 218–226.

29. Ebihara K., Tachibe M., & Kaneko N, et al. (2013). Hydroxypropylation of high-amylose maize starch changes digestion and fermentation-dependent parameters in rats. Journal of Nutrition Science, 2:1–881.

30. Mandalari G., Nueno Palop C., Bisignano G., Wickham M. S. J., & Narbad, A. (2008b) Investigation of the potential prebiotic properties of almond (Amygdalus communis L.) seeds. Applied Environmental Microbiology, 64, 264–4270.

31. Teixeira-Guedes, C., Sánchez-Moya, T., Pereira-Wilson, C., Ros-Berruezo, G., & López Nicolás, R. (2020). In Vitro Modulation of Gut Microbiota and Metabolism by Cooked Cowpea and Black Bean. Foods (Basel, Switzerland), 9(7), 861.

32. Wong, J. M. W., & Jenkins, D. J. A. (2007). Carbohydrate digestibility and metabolic effects. Journal of Nutrition, 137, 2539S-2546S.

33. Englyst, K. N., Englyst, H. N., Hudson, G. J., Cole, T. J., & Cummings, J. H. (1999). Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. American Journal of Clinical Nutrition, 69, 448–454.

34. Sheard, N. F., Clark, N. G., Brand-Miller, J. C, Franz, M. J., Pi-Sunyer, F. X.,Mayer-Davis, E., Kulkarni, K., & Geil. P. (2004). Dietary carbohydrate (amount and type) in the prevention and management of diabetes: a statement by the American Diabetes Association. Diabetes Care, 27, 2266–2271.

35. Case, L. P., Daristotle, L., Hayek, M. G., & Raasch, M. F. (Eds.). (2011). Chapter 2-Carbohydrates. In Canine and Feline Nutrition (3rd Edition ed., pp. 13-16). St. Louis, MO: Mosby Elsevier.

36. Tasie, O., Kassama, L. S., Mankolo, R., & Boateng, J. (2019). Instrumental Inductively Coupled Plasma Profiling of Mineral in Enzymatic Hydrolyzed Red Kidney Beans (Phaseolus vulgaris L.)". EC Nutrition 14(10), 893-901.

37. Day, C. N., & Morawicki, R. O. (2018). Effects of fermentation by yeast and amylolytic lactic acid bacteria on

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grain sorghum protein content and digestibility. Hindawi Journal of Food Quality, 2018, 1–8.

38. Sripriya, G., Anthony, U., & Chandra, T. S. (1997). Changes in carbohydrate, free amino acids, organic acids, phytate and HCl extractability of minerals during germination and fermentation of finger millet (Eleusine coracana). Food Chemistry, 58, 335-350.

39. Nkhata, S. G., Ayua, E., Kamau, E. H., & Shingiro, J. B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Science and Nutrition, 6(8):2446-2458.

40. Zhang, Y. Y., Panozzo, J., Hall, M. S., & Ajlouni, S. (2018). Bioaccessibility of Some Essential Minerals in Three Selected Australian Pulse Varieties Using an In Vitro Gastrointestinal Digestion Model. Journal of Food Science, 83(11), 2873-2881.

41. Blaine, J., Chonchol, M., & Levi, M. (2014). Renal control of calcium, phosphate, and magnesium homeostasis. Clinical Journal of the American Society of Nephrology, 10(7), 1257–1272.

42. Khanal, R. C., & Nemere, I. (2008). Regulation of intestinal calcium transport. Annual Review of Nutrition, 28(1), 179–196.

43. Zhang, Q., Xing, B., Sun, M., Zhou, B., Ren, G., & Qin, P. (2020). Changes in bioaccessibility, polyphenol profile and antioxidants of quinoa and djulis sprouts during in vitro simulated gastrointestinal digestion. Food Science & Nutrition, 8, 4232-4241.

44. Tagliazucchi, D., Verzelloni, E., Bertolini, D., & Conte, A. (2010). In vitro bioaccessibility and antioxidant activity of grape polyphenols. Food Chemistry, 120(2), 599–606.

45. Manach, C., Williamson, G., Morand, C., Scalbert, A., & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American Journal of Clinical Nutrition, 81(1), 230S–242S.

46. Parada, J., & Aguilera, J. M. (2007). Food microstructure affects the bioavailability of several nutrients. Journal of Food Science, 72(2), R21– R32.

47. Saura-Calixto, F., Serrano, J., & Goni I. (2007). Intake and bioaccessibility of total polyphenols in a whole diet. Food Chemistry, 101, 492–501.

48. Gan, R. Y., Deng, Z. Q., Yan, A. X., Shah, N. P., Lui, W. Y., Chan, C. L., & Corke, H. (2016). Pigmented edible bean coats as natural sources of polyphenols with antioxidant and antibacterial effects. LWT-Food Science and Technology, 73, 168–177.

49. Yadav, N., Kaur, D., Malaviya, R., Singh, M., Fatima, M., & Singh, L. (2018) Effect of thermal and non-thermal processing on antioxidant potential of cowpea seeds. International Journal of Food Properties, 21(1), 437-451.

50. Adetuyi, F.O., & Ibrahim, T.A. (2014). Effect of fermentation time on the phenolic, flavonoid and vitamin C contents and antioxidant activities of okra (Abelmoschus esculentus) seeds. Nigerian Food Journal, 232, 128–137.

51. Haile, M., & Hee Kang, W. (2020). Antioxidant properties of fermented green coffee beans with Wickerhamomyces anomalus. Fermentation, 6, 1-16.

PEER REVIEW

Not commissioned. Externally peer reviewed.

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FIGURES

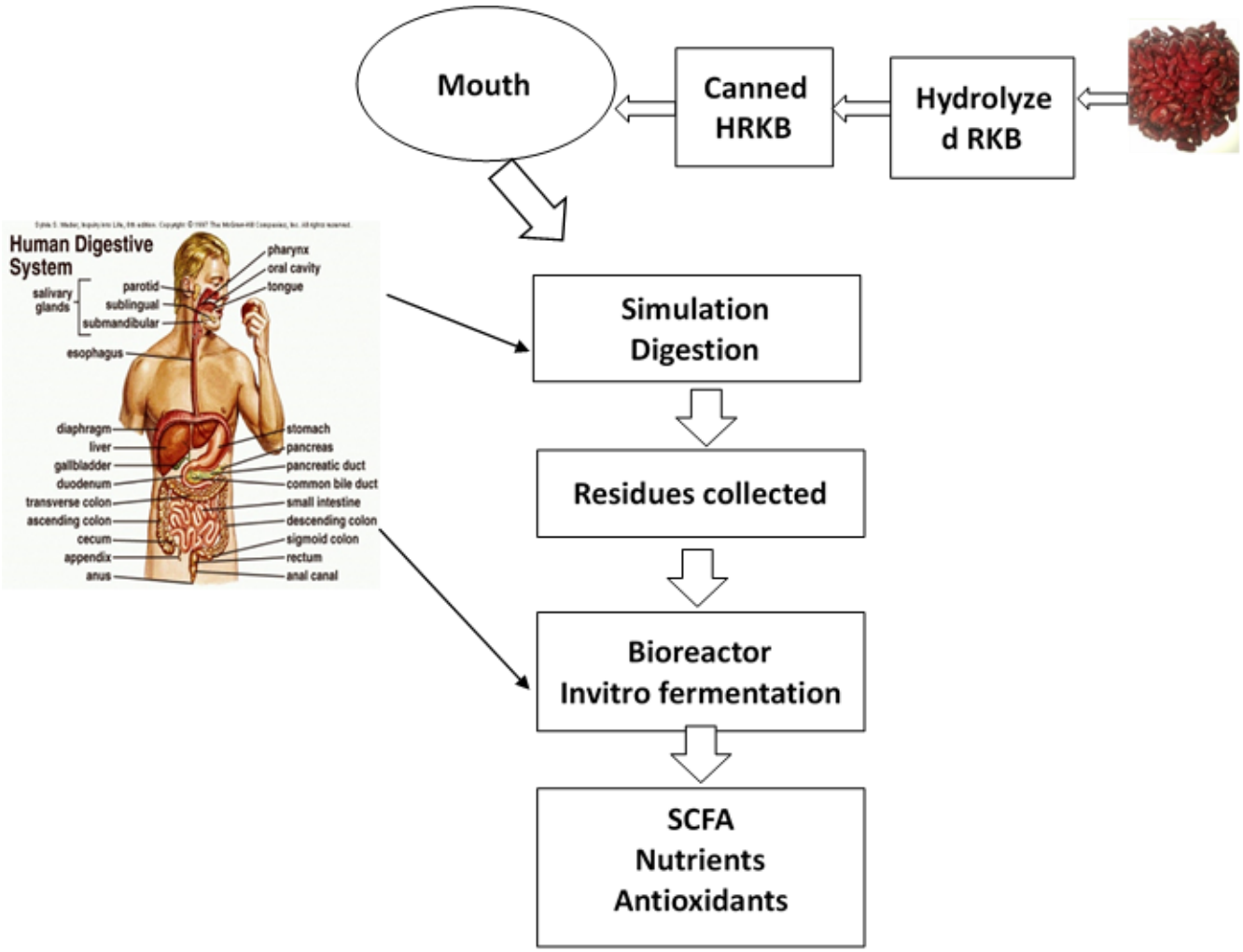

Figure 1. The System flow diagram of the simulation study of the digestion in the oral (mouth), stomach, and intestine phases.

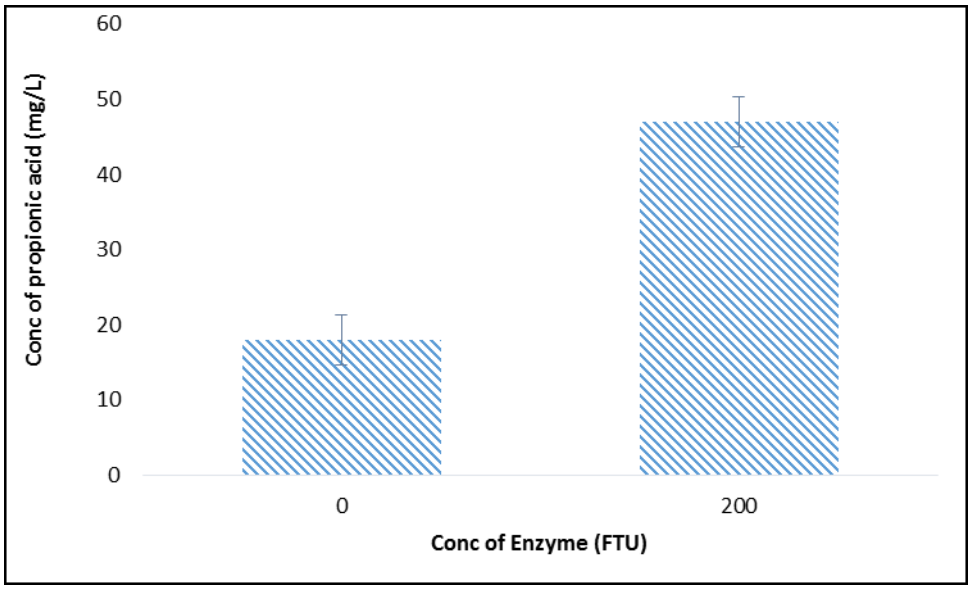

Figure 2. The Effect of Concentration of Enzyme (FTU) on the the concentration of PA in Hydrolyzed RKB.

(a)

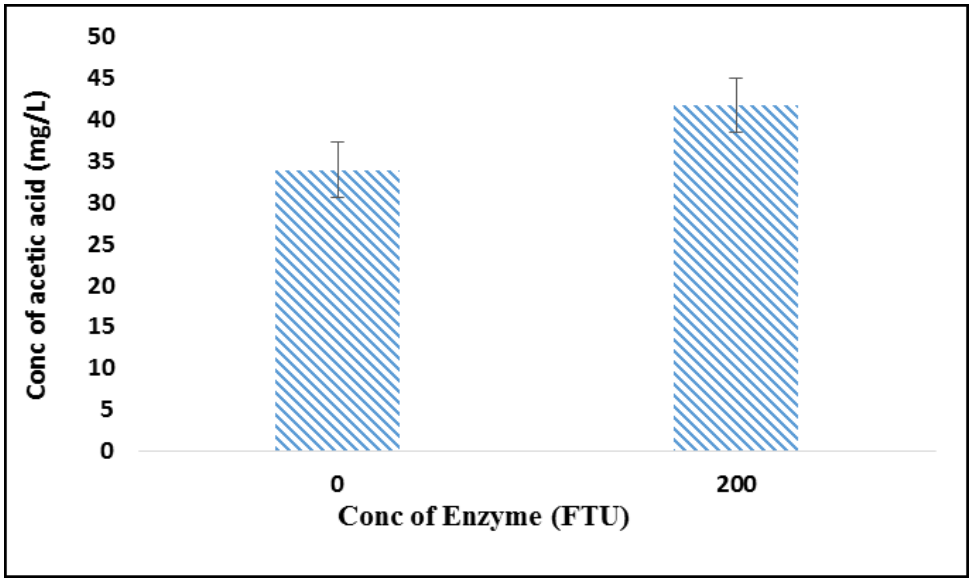

Figure 3. The Effect of Concentration of Enzyme (FTU) on the the concentration of AA in Hydrolyzed RKB.

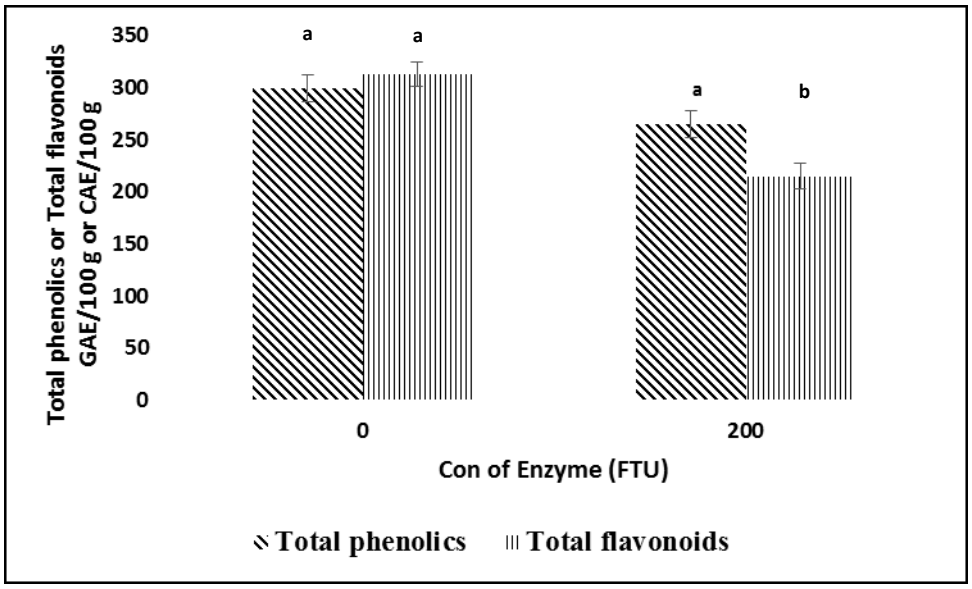

Figure 4. The mean GAE-Gallic acid equivalent standard TPC and CE- Catechin equivalent standard for TFC of Digested Canned RKB after *in vitro* fermentation at Enzyme Concentration of 0 and 200 FTU.

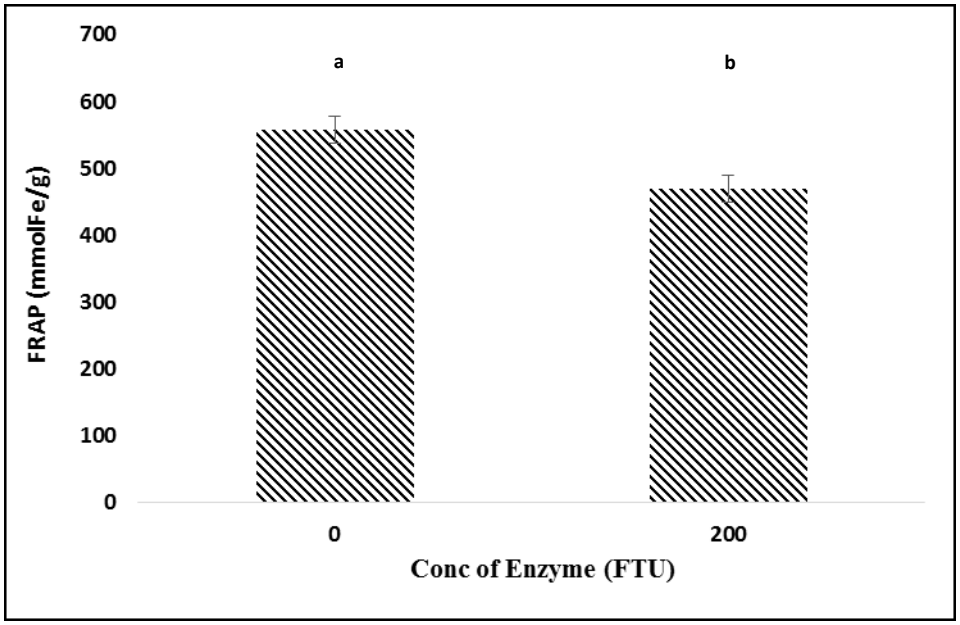

Figure 5. The mean FRAP of Digested Canned RKB at Enzyme Concentration of 0 and 200 FTU after in vitro fermentation.

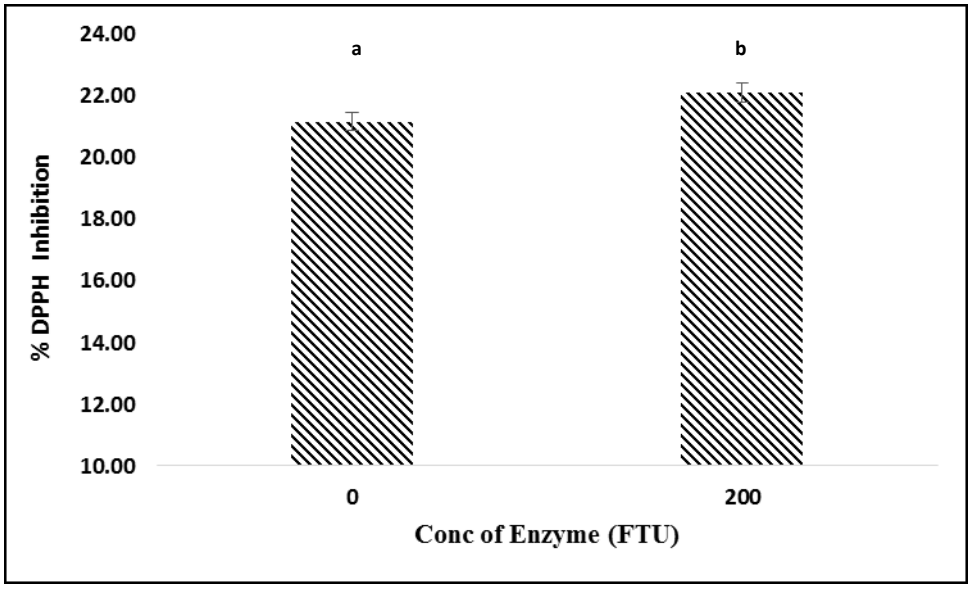

Figure 6. The Effect of Enzyme Concentration (FTU) on the % DPPH Inhibition of in vitro fermented RKB.

TABLES

Table 1. The mean (n = 3) mineral Content in raw RKB and Hydrolyzed canned RKB obtained before and after in vitro fermentation by ICP Analysis.

	Raw RKB (µg/L)	**After in-vitro fermentation (µg/L)**
Phosphorus	3184±88[a]	13997±230[a]
Magnesium	1025±17[b]	801±39[a]
Calcium	753±25[c]	634±28[a]
Iron	56±9[d]	75±10[b]
Zinc	52±2[d]	18±0.94[b]
Copper	4.8±0.4[d]	10.5±0.36[a]

Mean values with different letters within the same column show significant differences ($p \leq 0.05$) based on Duncan's multiple comparison test.