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Instrumental Inductively Coupled Plasma Profiling of Mineral in Enzymatic Hydrolyzed Red Kidney Beans (*Phaseolus vulgaris L.*)

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Abstract

Red kidney beans (RKB) contain essential minerals with significant health benefits. Thus, the bioavailability of these nutrients is impacted by anti-nutritional factors. Processing such as enzyme hydrolysis is one of the methods used to limit the effect of anti-nutritional factors. Hence, it was hypothesized that the enzyme hydrolysis of RKB could enhance the release of nutrients. The objective of this study was to quantify the mineral content in raw RKB, non-digested hydrolyzed canned RKB, and simulated digested canned RKB using the Inductively Coupled plasma 0ptical Emission Spectrometer (ICP-OES). Samples were freeze-dried and ground before microwave digestion using concentrated nitric acid. After digestion, the samples were filtered using a filter paper and diluted with deionized water. The aliquots collected after post-digestion of RKB were analyzed for mineral content (phosphorus, magnesium, calcium, iron, zinc, and copper) using the ICP-OES. The experiment was conducted in triplicates with $p < 0.05$ regarded as significant, using factorial design with two medium (brine and buffer solutions), and enzyme concentration (0 and 200 FTU). A high concentration of phosphorus (3184 mg/kg) in raw RKB; magnesium (1025 mg/kg) and lowest in copper (4.8 mg/kg) was observed. Phosphorus content was also higher (2371 mg/kg), as well as magnesium (772 mg/kg), and the lowest concentration observed in copper (6.8 mg/kg) in the non-digested hydrolyzed canned RKB. The phosphorus and magnesium concentration reduced significantly ($p < 0.05$) to 1651 and (482 mg/kg) respectively after simulated digestion phase. The brine solution enhanced the release of minerals at an enzyme concentration of 200 FTU. The result from this study shows that enzymatic hydrolysis can enhance the nutrient content in RKB.

Keywords: *Digestion; Canning; Hydrolysis; Legumes; Nutrients*

Abbreviations

ICP-OES: Inductively Coupled Plasma Optical Emission Spectrometer; RKB: Red Kidney Beans

Introduction

Red kidney beans (RKB) is a staple food for many, particularly in the developing countries, where alternate source protein is vital to meet the daily nutritional requirements. They are excellent and cheap sources of proteins, vitamins, minerals, fiber, and starch [1]. Red kidney beans earned its name 'poor man's meat' because of the high protein content; it significantly contributes to the prevention of malnutrition [2].

The high carbohydrate content (50 - 65%) in RKB is associated with slowing digestion with low glycemic index than other carbohydrates from rice, potatoes, and white bread [3]. Essential minerals are essential in the body to support various physiological and biochemical

processes [4] that occur in man and animals. Deficiency of micronutrients in the body can lead to impairment of brain function and mental development [5,6]. Hence, it is essential to consume foods rich in micronutrients to combat the deficits in nutrient requirements. Therefore, to establish a balance of minerals in the body, the amount of mineral that is bioavailable for biochemical processes in the body is very important [5].

Minerals from plant sources are not readily bioaccessible compared to animal sources because of the presence of antinutritional compounds. Antinutritional compounds such as phytic acid are prevalent in RKB, hence a factor that affects the bioaccessibility and potential efficacy of mineral utilization in the body [7]. The antinutritional factors are known to chelates minerals found in legumes and preventing their absorption and bioavailability [7]. Phytase enzymes are the well-known catalyst that hydrolyzes phytic acid dephosphorylate hence improving its bioaccessibility when consumed.

Phytase is chemically known as Myo-inositol hexakisphosphate phosphohydrolase that catalyzes the hydrolysis of phytic acid to free inorganic phosphate and other myo-inositol phosphates [8]. Phytase enzyme can be endogenous or exogenous. The phytase used in this study is an exogenous enzyme, Maxamyl. Temperature and pH value are the major factors that determine the enzyme activity. The Maxamyl phytase enzyme exhibits a pH range of 3 - 6 and a temperature range of 30 - 55°C. The removal of phytic acid increases the bioavailability of nutrients in food [9]. Phytase not only releases phosphorus, but also makes available other minerals such as magnesium, calcium; thus, making the released nutrients available for bone growth [10] and other vital health benefits.

Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was used to analyze minerals [11]. It has become a popular atomizer because it produces a chemically inert environment, produces very high temperatures, and provides a calibration range of several orders of magnitude [12]. ICP-OES has been used to analyze the concentrations of iron and zinc in common beans [13] and garden peas [14]. Also, Phan-Thien., *et al*. [12] detected essential minerals in peanut using ICP-OES. They stated that the results might be beneficial in determining peanut cultivars with enhanced micronutrient profile.

Many types of pretreatments before cooking, were adopted to reduce antinutritional factors in legumes, i.e. soaking, cooking, and blanching [15]. Lestienne., *et al.* [16] reported an increased *in vitro* solubility of iron and zinc by 2 - 23% after soaking cereal such as pearl millet with endogenous or exogenous phytase [9]. Betancur-Ancona [17] used ultrasonic pretreatment and subsequent extrusion to reduce antinutritional factors in RKB. The nutrient profile in canned RKB is rare in literature, and it is an essential information need for *in vitro* digestion study. Simulation digestion models were used to estimate the utilization of minerals in RKB simulating the gastrointestinal digestive system. It was hypothesized that hydrolyzing RKB with exogenous phytase enzymes (Maxamyl CENTERCHEM, Inc. Norwalk, CT) and canning will enhance the bioaccessibility of mineral during *in vitro* digestion. Hence, in this study, the effect of enzymatic hydrolysis cum canning process on the nutrient content (P, Mg, Ca, Fe, Zn, and Cu) of RKB during *in vitro* simulation was evaluated using the Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Materials and Methods

Sample preparation of raw RKB

The RKB was obtained from the Chippewa Valley Beans INC., Menomonie, Wisconsin. Upon receiving the samples, they were stored in a dark store at room temperature (21°C) until analysis. The RKB seeds were cleaned and inspected for cracks using a digital microscope (Model 12VDC 4 A, Motic Group Co. Vernon Hills, Illinois) and all cracked and non-visible potential damages seeds were removed, and the viable samples were sealed in plastic bags.

Enzymatic hydrolysis solutions

The food-grade phytase (MAXAMYL™ P) enzyme was provided by CENTERCHEM, Inc. Norwalk, CT is was derived from a selected strain of *Aspergillus niger*. The enzyme solutions were prepared a day before use using sodium acetate trihydrate at an enzyme concentration of 200 FTU and refrigerated at 4°C. The samples (100 g of RKB) were weighed and sealed in ziplock bags. Brine solution was prepared using sugar, salt, and CaCl [18] and the buffer solutions of sodium acetate, magnesium sulfate, and glacial acetic and 1 M glacial acetic acid was used to adjust the pH to 5.15 [19].

Enzymatic hydrolysis and commercial sterilization

The enzymatic hydrolysis process was done at the University of Georgia, Food Science, and Technology Pilot Plant. Standard can size 300 (300 x 407), and 100 g of RKB for each can was used for this experiment. Each can was filled with 334 mL of brine or buffer solutions either inoculated with 200 FTU as the treatment or no inoculation (0 FTU) known as the control. The cans were sealed using the Dixie Automatic Can Sealer (Model 23-500, Dixie Canner Co., Athens, Georgia). The canned samples were incubated in a reciprocating temperature-controlled water bath (Model 2872, Thermoscientific, Marietta, Ohio) for 6h and temperature-controlled at 37°C. The incubated cans were placed in the retort basket using a half-ton electrical operated chain hoist, and the basket was gently positioned inside the retort (Model RDSW-3SS, Serial no C-465, Dixie Canner Co., Jennings, Oklahoma). The cans were retorted 10 psi for 51 minutes and then cooled.

Simulation digestion

A simulation study of the digestive system was conducted according to the modified method described by [20]. The canned RKB were randomly selected from each treatment groups' (0 FTU brine, 0 FTU buffer, 200 FTU brine, and 200 FTU buffer) solutions. The oral digestive system was simulated using 30g of the selected canned RKB samples and placed into a flask and homogenized in 30 mL saline solution using a tissue tearor (Model S85-370, Biospec Products, Inc., Bartlesville, OK). Sodium chloride mixed with urea and α- amylase (40 µL) and incubated for 2 minutes was used as salivary fluid (24 mL) added to the samples to simulate oral digestion.

In the simulated gastric digestive system, the pH of the RKB digesta from the oral phase was adjusted to 2 using 1M HCl. Pepsin (1.875 mL) was added to the digesta in 250 mL flasks and incubated at 37°C in a reciprocal shaking bath (Model 2872, Thermoscientific, Marietta, Ohio), agitated at 70 rpm for 2h, hence ends the digestive phase.

After the gastric digestion phase was completed, to simulate the intestinal digestive phase the pH of the digesta was adjusted to 7 using 1 M $NaHCO_3$ and 3 mL of the pancreatin-bile salt mixture was added to each 250 mL flask containing the digesta and incubated for 2h. The enzymes were deactivated by heating the samples at 70°C for 20 minutes in a reciprocal shaking water bath (Model 2872, Thermoscientific, Marietta, Ohio) and immediately refrigerated overnight. The digesta were then centrifuged with the Sorvall legend centrifuge (Model XTR, Thermofisher Scientific, Ridgefield, CT) at 3000g at 25°C for 15 minutes. 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) in preparation for the ICP-OES analysis.

Quantification of the mineral content of hydrolyzed RKB

Microwave digestion of RKB samples

The digesta (residue) recovered from the simulation of the GIT and raw RKB and non-digested hydrolyzed canned RKB) freeze-dried using virtis genesis pilot lyophilizer (Model# 25L Genesis SQ Super XL-70, Stone Ridge, New York). The dried samples ground using a coffee grinder and 0.5g of the ground samples were placed in test tubes in duplicates, and 10 mL of concentrated nitric acid was carefully measured and added to in each test tube. Nitric acid was used as the blank. The samples were carefully arranged in the microwave basket before transferring them to the microwave digester (CEM MarsXpress, Model# MD7330, Matthews, North Carolina). The samples were digested using the Plant tissue 40 express methods at a maximum power of 1200 W for 15 min at 200°C and subsequently cooled under the hood. The digested samples were gently opened under the hood and filtered with Whatman filter paper (Filter #43). The volume of the filtrate was diluted to 25 mL using deionized water stored in the refrigerator until further analysis.

Instrumental analysis of the mineral content

The filtrate collected from the microwave digestion were analyzed using the ICP-OES (Model Optima 2100DV, PerkinElmer Inc., Wellesley, MA). Predetermined methods were programed into the ICP-OES system, and the elements Mg, Ca, Cu, Zn, P and Fe were analyzed. The standards and the blank were placed in their correct positions. The quality control (QC) was checked for system adequacy, and the samples were run through the analyzer, and the results were obtained via a computerized data logger.

Results

Mineral content in raw red kidney beans

The results of the ICP-OES mineral analysis of raw RKB is shown in figure 1. The phosphorus (P), Mg, and Ca were significantly different ($p < 0.05$) from the Fe, Zn and Cu content obtained via the ICP-OES analysis of raw RKB. The P content was 3184 mg/kg, hence marked the highest in the raw RKB, followed by Mg (1024 mg/kg). Onglo., *et al*. [6] reported 4159 mg/kg and 1241.7 mg/kg for P and Mg in raw beans, respectively.

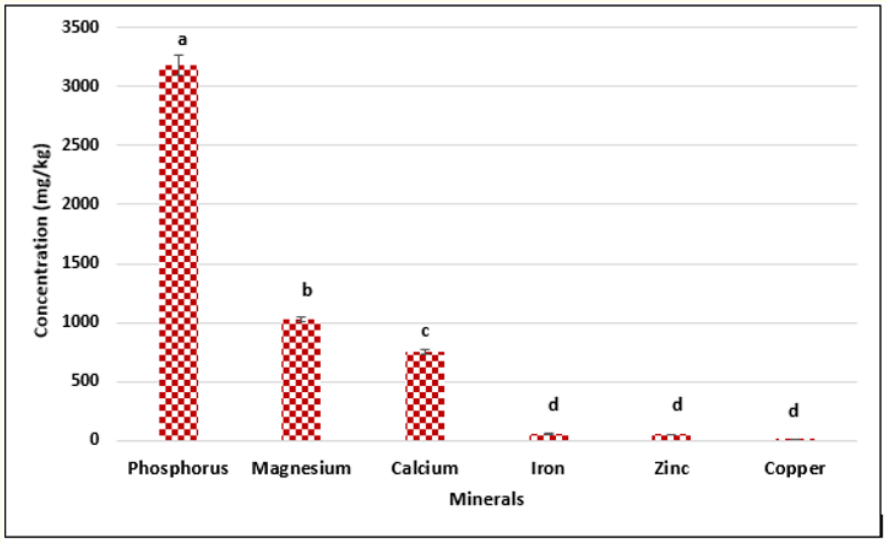

Figure 1: *The mean mineral profile in Raw RKB obtained through instrumental ICP analysis. Means with different alphabets show a significant difference ($p \leq 0.05$) in mineral concentrations in RKB by Duncan's mean comparison test.*

The Ca content reported in this study for the raw RKB was 753 mg/kg [21] stated a range 550 to 1500 µg/g in lentils, 1023.7 µg/g in white beans, and 1125 mg/kg reported by [6] in boiled beans. The Fe content in white beans reported by [21] was within the range of 28 to 69.3 µg/g, which is much higher than the value (56 mg/kg) observed in this study on RKB; hence, [21] reported 66.3 µg/g in white beans and 47.8 µg/g in chickpeas. Timoracka., *et al*. [22] reported 64.87 to 121.17 mg/kg in kidney beans. The differences could be attributed to variety and procedural differences.

The Zn content in white beans was noted as 28 - 56 µg/g [23], 34.5 in white beans and 43.8 in chickpeas [21] and 52 mg/kg in RKB was observed in this mineral profile study. Comparatively [22] reported Zn content in the range of 26.65 to 37.35 mg/kg in kidney beans and 22.29 to 37.68 mg/kg was reported by [24] in raw common beans.

The Cu content ranging from 7.72 to 11.38 mg/kg in RKB was reported by [22]. Nestares., *et al*. [25], reported 1.65, 3.85, and 1.62 g/kg for Ca, P, and Mg content in raw beans. Zhang., *et al*. [26] reported Mg content in Lupin to be 217.46 mg/100 g, Ca (113.56 mg/100), P (496.98 mg/100), Zn (4.86 mg/100g) and Cu (0.55 mg/100). Hayat., *et al*. [27] stated in their work that RKBs are good source of minerals. However, the Cu content in this study was recorded 4.87 mg/kg via ICP-OES analysis in raw RKB.

Mineral profile in hydrolyzed canned red kidney beans

Red kidney beans were enzymatically hydrolyzed in brine or buffer solutions, and the results of the ICP-OES analysis of the mineral profile is shown in figure 2. Reduced mineral (P, Mg, Ca, Fe, and Zn) concentrations were observed on the ICP-OES profile of enzymatic hydrolyzed and canned RKB. A reduction of about 25% was observed on P and Mg, 10% on Ca, 37% in Zn, and 40% increase were observed in Cu. Brigide., *et al*. [24] reported Zn concentration range of 20.81 to 34.76 mg/kg in cooked legumes. The Fe content of hydrolyzed canned RKB was 34 mg/kg. In a study conducted by [28], a decrease (0.65 mg/100g) in Zn content after pressure and microwaving cooking of the whole cowpea and a high concentration in whole raw cowpea (1.21 mg/100g) was observed. Comparatively, in this study, a reduced Zn content was detected after canning. Jing., *et al*. [28] also reported an increase in Fe content after microwave cooking of whole chickpea (0.79 mg/100g) and low concentration in whole raw chickpea. Low mineral profile was observed in the canned hydrolyzed red kidney beans; this difference could be procedural. However, some factors could be responsible for the differences in the nutrient content [29], for examples cell structure and protein complexes with other constituents coupled to the localization of nutrient within the seed structure [29].

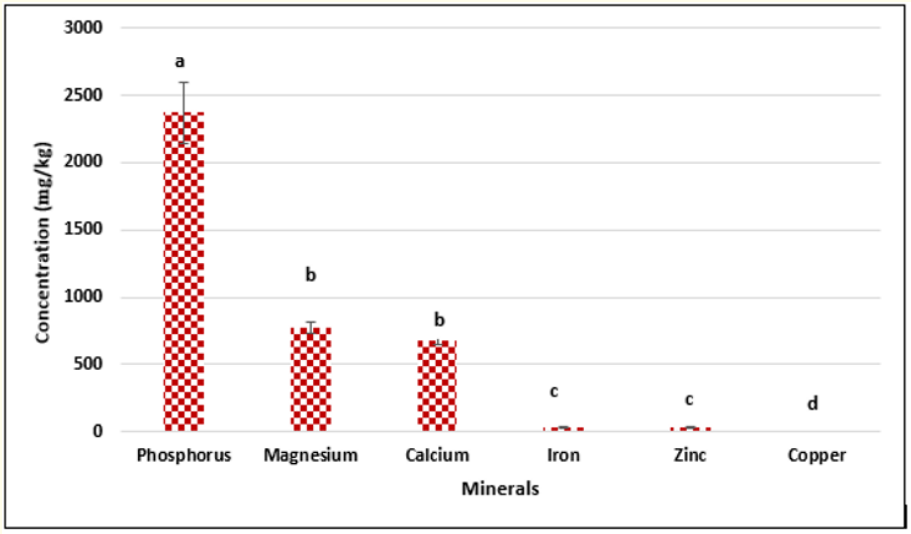

Figure 2: *The mean mineral profile in enzymatic hydrolyzed and canned RKB obtained through instrumental ICP analysis. Means with different alphabets show a significant difference ($p \leq 0.05$) in mineral concentrations in RKB by Duncan's mean comparison test.*

Mineral Profile in digested hydrolyzed canned red kidney beans

Figure 3 shows the mineral profile after the *in vitro* simulation of the digested hydrolyzed canned RKB residues. The P and Mg content was observed to decrease significantly ($p < 0.05$) after digestion. No significant difference ($p > 0.05$) was observed in Ca content after the *in vitro* digestion. The Fe content after digestion of hydrolyzed canned RKB was 260 mg/kg. Beiseigel., *et al*. [30] reported high Fe uptake in Caco-2 cell after *in vitro* digestion of cooked pinto beans compared to the non-digested. Zinc was detected in the ICP-OES profile of the residue; hence, that could be an indication of bioaccessibility and the absorption of Zn during the digestion phase. Admassu [31] reported Zn value ranging from 15.39 to 28.03 mg/kg; hence, [32] indicated the binding of Zn by phytic acid might have decreased the availability, absorption, and re-absorption in the GIT. The enzymatic hydrolysis process may have freed the Zn from the phytic acid bond and increased the absorption during the digestion and; thus, resulting in none detection on the post-digestive residue.

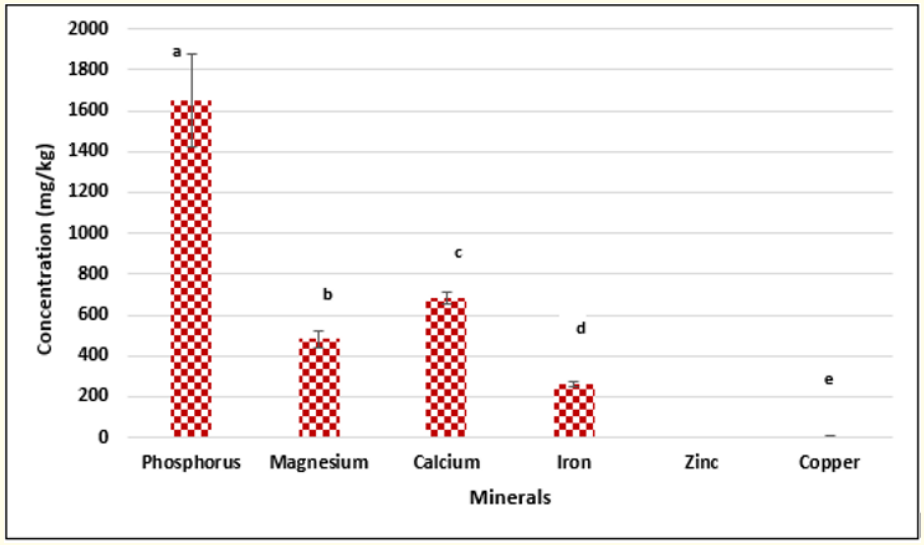

Figure 3: *The mean mineral profile in in vitro digested enzymatic hydrolyzed, and canned RKB obtained through instrumental ICP analysis. Means with different alphabets show a significant difference ($p \leq 0.05$) in mineral concentrations in RKB by Duncan's mean comparison test.*

Discussion

This study has shown the mineral profile of raw RKB, hydrolyzed and canned RKB and residue of hydrolyzed and canned RKB after digestion analyzed using ICP-OES. Many research has reported the mineral content of different varieties of beans [5,24,26]. The variation of mineral content could be attributed to many intrinsic factors that influence the variability of mineral content in beans. Antinutritional factors such as phytic acid and tannins are typical factors in beans and legumes that contribute to the variability of minerals [5]. Nestares., *et al*. [25] reported Ca to be the most abundant in soaked beans, and insignificant losses were also observed. The different antinutritional factors present in beans may have contributed to the bioaccessibility and absorption when consumed [5,33]. Similarly, minimal loss of Ca was observed in hydrolyzed canned RKB and hydrolyzed canned digested RKB, hence high concentrations were recorded in this study.

Furthermore [25] indicated that the processing of the legumes tends to modify the critical components that may impact the absorption of Ca. The Ca content in raw RKB was much higher, and this study shows the enzymatic hydrolysis-cum- canning may have enhanced the bioavailability in the RKB residue after the *in vitro* simulation of the digestive system. The Maxamyl phytase enzyme used in hydrolyzing the RKB may have minimized the chelation of minerals P, Ca, Mg, and other minerals detected in the ICP-OES analysis.

According to [34], phytic acids are broken down to inorganic P and other inositols by the enzyme phytase present in the legume during digestion. The exogenous phytase enzyme (Maxamyl) used in the study acting in synergy with the endogenous enzymes may have impeded the phytic acid chelation of minerals. Nestares., *et al*. [25] stated that the digestive utilization of P was acceptable for growing rats used in their study because the pH was moderate acidic (5.6) which was optimum for phytase activity in the beans; thus produced a more significant amount of absorbable phosphorus [25]. In our work, the buffer solution was adjusted to a pH of 5 used as one of the treatments. However, we observed in this study that more Fe concentrations were detected in the simulated digesta than in the canned hydrolyzed RKB.

Conclusion

The ICP mineral profile analysis shows the highest P concentration of 3184 mg/kg, Mg (1025 mg/kg) and Cu (4.8 mg/kg) was the lowest observed. The P content was also higher (2371 mg/kg), Mg (772 mg/kg), and the lowest concentration was observed in Cu (6.8 mg/kg) in the non-digested hydrolyzed canned RKB. The P and Mg concentration reduced significantly ($p < 0.05$) to 1651 and 482 mg/kg respectively after simulated digestion phase. The brine solution enhanced the release of minerals at an enzyme concentration of 200 FTU. This study shows that enzymatic hydrolysis can enhance the nutrient content in RKB. The hydrolyzed canned RKB assayed showed limited post-processing (canning) effect on the mineral profiles; this further confirms the reports that red kidney beans are good sources of minerals. After the *in vitro* digestion, minerals were also present except zinc.

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Conflict of Interest

None.

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