#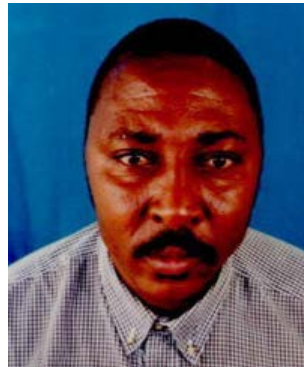 PHENOLIC COMPOUNDS, PHYTATE, CITRIC ACID AND THE *IN-VITRO* IRON ACCESSIBILITY OF COWPEAS, MUNG BEANS AND FOUR VARIETIES OF KIDNEY BEANS

COMPOSES PHENOLIQUES, PHYTATES, ACIDE CITRIQUE ET LE FER ACCESSIBLE IN-VITRO DE NIEBES, HARICOTS MUNGO ET QUATRE VERIETES DE HARICOTS ORDINAIRES

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ABSTRACT

Iron deficiency anaemia is highly prevalent in Tanzania affecting predominantly children and women of childbearing age. One of the major causes is the low iron bioavailability from vegetarian diets mainly due to the presence of various antinutritional factors that interfere with non-heme iron absorption. Cereals and legumes constitute the main ingredients of diets in the country providing proteins, carbohydrates, minerals and vitamins. Certain varieties of these grains contain large amounts of polyphenolics and phytate that are known to inhibit iron absorption. Varieties of legumes; cowpeas (Vigna unguiculata) and mung beans (Vigna radiata L.) and kidney beans (Phaseolus vulgaris L.) were analyzed for the polyphenolics and phytates. The total and *in vitro* accessible iron, and the citric acid were also quantified and their nutritional consequences discussed. Phenolic compounds varied widely in the analysed legumes ranging from 3.37 to 9.14 mg catechin equivalent/g and they associated negatively with *in vitro* accessible iron (r = - 0.367; p = 0.054). The catechol and resorcinol phenolics ranged from 1.58 to 3.51 and 1.41 to 5.37 mg catechin equivalent/g respectively and were relatively higher than galloyls that range from 0.10 to 1.52 mg tannic acid equivalent/g). Phytate ranged from 8.46 to 13.18 mg/g, total iron from 3.58 to 7.55 mg/100g and *in vitro* accessible iron from 0.45 to 1.04 mg/100g. Citric acid ranged from 70.8 to 205.2 mg/100g and was associated positively with *in vitro* accessible iron (r = 0.845; p = 0.006). Proper processing of legumes to reduce antinutritional factors to relatively lower levels is important in order to render the iron and other nutrients readily available for absorption.

Keywords: Phytate; phenolic compounds; galloyls; catechols; resorcinols; citric acid; *in vitro* accessible iron

[French] RESUME

L'anémie ferriprivée est fortement répandue en Tanzanie touchant principalement, les enfants et les femmes en âge de procréer. L'une des principales causes est le faible bio-disponibilité en fer des régimes végétariens due principalement de divers facteurs antinuritionnelles qui empêchent l'absorption de fer-non-hémique. Les céréales et les légumes constituent la source principale d'alimentation dans ce pays. Ils fournissent les protéines les hydrates de carbone, les minéraux et les vitamines. Les variétés de légumes ; des niébés, (vigna unguiculata) et haricots mungo (vigna radiata L) et des haricots ordinaires (phaseolus vulgaris L) ont été analysées pour voir leur contenance en polyphénoliques et phytates. L'ensemble le fer accessible in-vitro et l'acide citrique ont été également quantifiés et leur impact nutritionnel discuté. Les résultats de l'analyse ont indiqué que les composés phénoliques ont largement varié allant de 3,37 à 9,14 mg équivalent/g de catéchine et ils se sont associés de façon négative au fer accessible in-vitro (r=-0,367; P=0,054) La contenance en catéchol et résorcinol (composés phénoliques) ont varié respectivement de 1,58 à 3,51 et de 1,41 à 5,37mg de catéchine équivalent/g respectivement et étaient des taux relativement plus élevés que celui de galloys qui a varié de 0,10 à 1,52mg d'acide tannique/g. Les phytates ont varié de 8,46 à 13,18mg/g, le fer total a varié de 3,58 à 7,55 mg /100g et le fer accessible in-vitro de 0,45 à 1,04 mg / 100g. La teneur en acide citrique a varié de 70,8 à 205,2 mg / 100g et a été associé positivement avec le fer accessible in-vitro (r= 0,845g;p=0,006) Il est important qu'un traitement approprié des légumes soit réalisé pour réduire les facteurs anti-nutritionnels à des niveaux relativement bas afin de faciliter l'absorption du fer et d'autres nutriments.

Mots clefs : phytates, composés phénoliques, galloys, catéchols, résorcinols, acide citrique, fer accessible in-vitro.

INTRODUCTION

Legumes are extensively consumed in Tanzania like in other African and less developed countries. Production of legumes (beans) in Tanzania is estimated at above 289,700 tones a year. It is mostly consumed as cooked whole dry seeds taken as a relish with cereal staples. They are also used in the formulation of simple weaning blends with cereals, which are relatively cheap for the poor rural to afford. These grains are important sources of protein, calories, vitamins and minerals for the country's population. However, the nutritional quality of these grains may be impaired due to the presence of antinutritional factors such as phytate, polyphenols, trypsin inhibitors and flatulence causing oligosaccharides [1-3]. The presence of these antinutrients may cause low protein digestibility and mineral availability in legumes. Cowpeas have been shown to contain high level of polyphenols, which play an important role in the reduction of protein digestibility and starch digestibility [4,5]. Condensed tannins, widely distributed in cereals and legumes, have been reported to impair iron availability [6-8]. Likewise, phytate compounds in food grains have been reported to lower the bioavailability of minerals like iron [9,10]. Iron deficiency anaemia has been shown to have high association with low iron availability from vegetarian diets and polyphenols and phytate are likely to account for the poor iron accessibility in Tanzanian diets [11]. Iron deficiency anaemia contributes significantly to the prevalence of anaemia in Tanzania [11]. Knowledge on the distribution of these antinutritional factors in Tanzanian food might provide the proper ways of pre-treatment and processing of the diet ingredients for the improvement of their quality.

The objectives of this study were to determine the amount of antinutritional factors such as phytate and polyphenolic groups of legume grains widely consumed in Tanzania. To determine the total iron content and *in vitro* iron accessibility as well as the content of citric acid of these legumes and describing their nutritional consequences.

MATERIALS AND METHODS

Materials

Cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata* L.) and varieties of common edible kidney beans (*Phaseolus vulgaris* L.) with distinct color differentiation (red, brown, reddish-brown and yellow) were obtained from local markets in Morogoro and Dodoma regions in Central Tanzania. The grains were purchased randomly from retail sellers in the market in lots of one kilogram. The dry grains were sorted and washed by distilled water at room temperature to remove foreign particulates and dust before being milled and analyzed. The chemicals and reagents; citric acid (C0759), catechin (C1788), tannic acid (T0125), pepsin (P1750), pancreatin (P6887) and bile extract (B8631) were purchased from Sigma, Sweden.

CHEMICAL ANALYSIS

Proximate Composition

Moisture, crude protein (Micro-Kjeldahl, Nx6.25) and crude fat were determined by standard methods [12].

Determination of Citric Acid

The concentration of citric acid was determined using HPLC [13]. The chromatograph instrumentation consisted of a pump (Waters Assoc., model 510), U.V. detector operating at 210 nm (Lambda HP 1050), column heater & controller and an Aminex HPX-87H (BioRad). Injection volume was 60 µl and sample dilution was 1/20. The acid was eluted at 65oC using 0.008M H_2SO_4 as the mobile phase and flow rate of 0.6 ml/min. The concentration of citric acid was determined by reference to the elution of standard citric acid.

Determination of Phenolic Compounds

The content of total phenolics was determined by the Prussian blue test method according to Price and Butler using acidified methanol as extraction solvent [14]. Resorcinol phenolic groups were determined by the modified vanillin method with blank subtracted using acidified methanol as extraction solvent [15]. The catechol and galloyl phenolic groups were determined by employing the ferric ammonium sulphate (FAS) method as described by Brune, Rossander and Hallberg with minor modifications in the extraction procedure [6]. A 50% Dimethyl-formamide in acetate buffer (pH 4.4) was used as extraction solvent. Sample extraction for the above analyses was made by mixing 200 mg of the sample flour with 5 ml of the extraction solvent and homogenized for one hour with occasional shaking at an interval of 10 minutes. The mixture was centrifuged at 5000 g for 15 minutes and the supernatant collected. The extraction was repeated once and the supernatants were pooled together and analysed for different phenolics. The values are expressed in mg catechin equivalent/g sample for total phenols, resorcinols and catechols and in mg tannic acid equivalent/g sample dry weight for galloyls.

Determination of Phytate

Phytate compounds were determined by the method described by Carlsson et al. [16]. A 0.5 g sample of the flour was extracted with 10 ml of 0.5 mol/l HCl over night followed by centrifugation at 3500 g for 5 minutes and filtered. The supernatant was collected and analysed for total phytate content by High-performance ion chromatography (HPIC). The values are expressed in mg/g (dry wt).

Determination of total iron and *in vitro* iron accessibility

The total iron content was determined by wet digestion of the sample in a microwave system (Milestone Bergano, Italy). About 0.3 g of the flour was mixed with 3.0 ml of water, 0.15 ml of conc. HCl and 0.75 ml of conc. HNO_3 and digested in the microwave followed by cooling and diluting the mixture with water to 10 mL volume. The total iron was measured in the solution by HPIC analysis as described by Fredrikson et al.[17]. The determination of iron accessibility at physiological condition was done according to the method described by Svanberg, Lorri and Sandberg with minor modification [7,18]. 1.0 g of the flour was suspended in 10 ml= of distilled water and 10 ml of 0.3% pepsin solutions (in 0.1 M HCl) was added and digested for 90 minutes at 37oC. The pepsin solution contained physiological amounts of Na (49 mmol/l as NaCl), K (12 mmol/l as KCl) , Ca (10 mmol/l as $CaCl_2.2H_2O$) Mg (2.4 mmol/l as $MgCl_2$) and phosphate (3.5 mmol/l as KH_2PO_4). The pH was adjusted to 2.0 by 1.0 M NaOH and added 3 ml of pancreatic (0.012 g) and bile (0.075 g) solutions in 0.1 M $NaHCO_3$ before adjusting the pH to 5.0. The mixture was incubated for another 30

minutes and then adjusted the pH to 6.0 before centrifuging at 5000 g for 20 minutes. The supernatant was filtered through 45µm filters and the soluble iron determined by atomic absorption spectrometry (Pye Unicum, Sweden).

RESULTS

Crude Protein and Crude Fat Content

Crude protein content ranged from 18.8 to 21.9% and crude fat content ranged from 1.7 to 2.9% (Table I). The protein and fat compositions of the analysed grains were within the normal range of leguminous grains [19,20].

Citric Acid Content

Citric acid content in the legumes ranged from 70.8 mg/100g (mung bean) to 205.2 mg/100g (kidney bean redish/brown variety) (Table 1). Mulyowidarso et al.[13] reported values of 124 mg/100g citric acid in soybean and Wills et al.[21] reported 110 mg/100g citric acid in yard-long beans. Addition of organic acids, commonly found in vegetables, like citric acid was shown by Gillooly et al.[22] to improve the geometric mean of iron absorption from a basic rice meal in human studies. The same group further observed that the vegetables that were associated with moderate or good iron bioavailability contained appreciable amounts of one or more of the organic acids; malic, citric and ascorbic acids.

Phenolic Compounds

Phenolic compounds varied widely in the analysed grains (Table 2). Total phenolics ranged from 3.47 to 9.14, catechols from 1.58 to 3.51 and resorcinols from 1.41 to 5.37 mg catechin equivalents/g sample. Galloyls were found in lower amounts ranging from 0.10 in yellow kidney bean varieties to 1.52 mg tannic acid equivalents/g in cowpea varieties. Cowpeas contained the highest amount of total phenolics as well as phenolic groups and yellow kidney beans contained the least (Table 3). The wide variation observed in phenolic content is reported elsewhere [2,23]. Preet and Punia, [4] reported total phenolics in cowpea ranging between 7.79 to 9.35 mg catechin equivalent/g [4]. Structural grouping of phenolics into resorcinols, catechols and galloyls is less reported [18]. Catechol and resorcinol contents were relatively higher than galloyls in the analyzed legumes. However, galloyl and catechol phenolic groups are among the types of phenolics that have been reported to have high inhibitory effect to iron absorption [6,24,25,26]. Glahn et al.[24] reported 92% inhibition of iron uptake at low concentration of 1:0.1 iron: tannic acid molar ratio in model systems. Since galloyl and resorcinol phenolic groups have been associated with inhibitory effect on iron absorption, these phenolic groups in the analyzed legumes are therefore likely to cause potential inhibitory effects to the *in vitro* accessible iron. Inhibitory effects of polyphenols to *in vitro* iron accessibility as well as iron availability in humans have been reported [6,18,21,25,27]. Udayasakhara-Rao[8] reported a negative correlation (r= - 0.52) between tannin content and ionizable iron content of ungerminated and germinated groundnuts. Benitez, Grijalva and Valencia [20] reported negative association between iron accessibility in tepary beans (P. *acutifolius*) and the compounds of tannin, oxalates and phosphates .

Phytate Compounds

Phytate content ranged from 8.46 (mung bean) to 13.18 (cowpeas) mg/g sample (Table 2). Different amounts of phytate in pulses and legumes have been reported [28,29]. Preet and Punia, [4] reported amount of phytic acid in cowpea varieties as ranging from 8.19 to 9.50 mg/g and Vijayakumari et al.[23] reported values ranging from 3.20 to 4.80 mg/g in two species of *Vigna* seeds. Alonso et al. [9] reported concentrations of 4.90 and 5.10 mg/g phytic acid, in raw kidney beans and peas, respectively. The observed concentrations of phytate compounds in this study are high enough to form a strong complex with iron and therefore can lower the *in vitro* accessible iron [10,24,27,29].

Total and *in vitro* Accessible Iron

Total iron ranged from 3.58 to 7.55, with a mean of 5.46 mg/100g and the *in vitro* accessible iron ranged from 0.45 to 1.04 with a mean of 0.69 mg/100g. The percentage soluble iron ranged from 10.8% to 12.9% (Table 3). The mean total iron content in these grains were low compared to those reported by Benitez, Grijalva and Valencia, [20] in tepary (P. *acutifolius*) and pinto beans (P. *vulgaris*) and those reported by Saikia, Sarkar and Borua, [19] in rice bean (*Vigna umbellata*). Amounts of total iron close to this study have been reported in legumes [8,28,29]. The *in vitro* accessible iron (mg/100g) as well as their percentage reported in this study were less than those reported by Tatala, Svanberg and Mduma [11], however, in the latter study the legume samples were cooked. The *in vitro* accessible iron was, however, higher compared with amount reported for cereals [11,18]. Benitez, Grijalva and Valencia, [20] reported amount of soluble iron in tepary (P. *acutifolius*) and pinto beans (P. *vulgaris*) ranging from 0.319 to 0.965 mg/100g.

DISCUSSION

The association between the *in vitro* accessible iron and citric acid and total phenolics are illustrated in Figure 1.
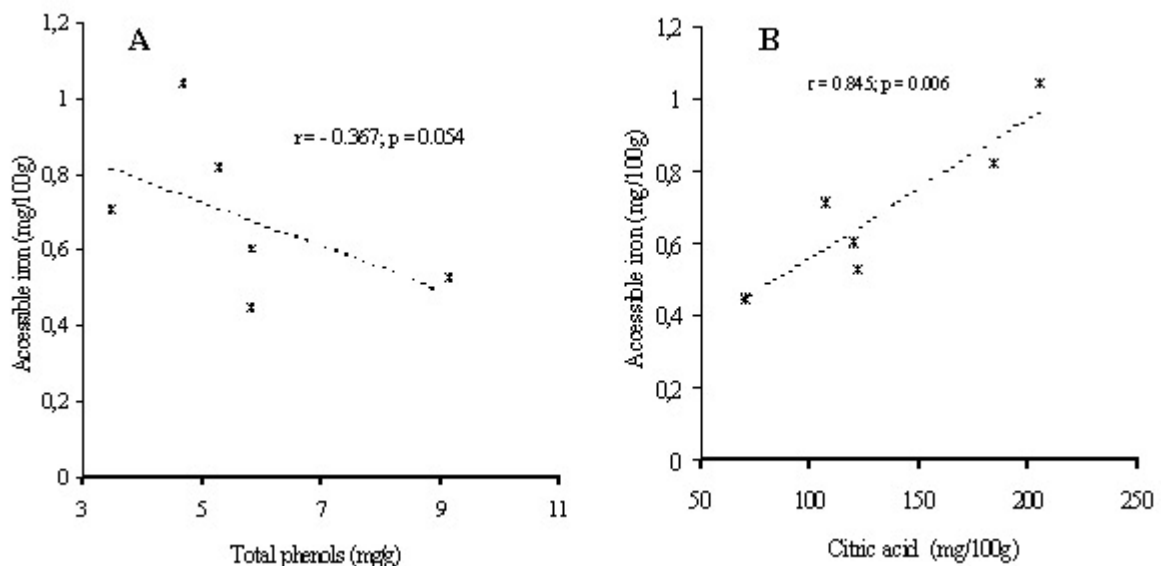

Figure 1: Relationship between in-vitro accessible iron and (a) total phenolics and (b) citric in legumes

The higher amounts of citric acid might have enhanced the *in vitro* accessible iron [22]. Citric acid associated with *in vitro* accessible iron positively when multiple regression analysis was conducted (r = 0.845; p = 0.006). Organic acids in vegetarian diets are potentially involved in influencing iron absorption. This effect can be more or less marked, depending on the processes to which the foods will be subjected. On the other hand, polyphenolic compounds might have lowered the *in vitro* accessible iron [6,18,22]. The total phenolic compounds were associated with *in vitro* accessible iron negatively when using the multiple regression analysis (r = - 0.367; p = 0.054). Phenolic groups gave a similar effect to that of total phenolics on the *in vitro* accessible iron. The phenolic groups gave negative correlation coefficient with the *in vitro* accessible iron. The correlation coefficients between the *in vitro* accessible iron and the phenolic groups were - 0.55 with galloyls, - 0.34 with catechols and - 0.33 with resorcinols. Polyphenols are a powerful factor affecting iron availability from vegetarian foods. Phytate compounds are another factor that affect iron availability from vegetarian foods [22,27]. In this study we failed to find a direct relationship between phytate and the *in vitro* iron accessibility. The legume samples assessed however, contained appreciably high amounts of phytate that might have inhibited the *in vitro* accessible iron. The range reported in this study for phytate compounds (8.46 to 13.18 mg/g) is relatively high than thus reported by Vijayakumari et al. [23] and thus reported by Alonso et al.[9].

CONCLUSION

In general, non-heme iron absorption has an important role in vegetarian diets. In this study polyphenolic compounds were shown to have a negative effect on the *in vitro* accessible iron from various legumes. Also the phytate content in all samples analyzed was observed to be high enough to strongly affect the *in vitro* accessible iron. On the other hand, the citric acid was high in these legumes and had an enhancing effect on *in vitro* accessible iron. Existence of other intrinsic compounds in the analyzed grains like fibers and elementary compounds that interact differently with iron and/or the antinutritional compounds may also contribute to variations observed in the *in vitro* accessible iron. This study emphasizes the particular importance of organic acids in vegetarian foods on the enhancement of dietary iron absorption. The observed high amount of inhibitors of iron absorption in the legumes calls for proper processing and/or pre-treatments of these grains that will reduce these compounds to levels that will render the nutrients readily available for absorption in the body. The processing methods should also leave the important factors like citric acid less affected. Decortication, germination and fermentation are among the processing methods that are earmarked. Enzymic treatments like use of polyphenol oxidase (PPO) for the degradation of polyphenolics and phytase for the degradation of phytate compounds can also be used in combination with the above processing methods. Another measure is to change the eating habits; this will ensure availability of enhancing factors in the diet or addition of organic acids like citric acid during preparation of the food, particularly complementary foods. Further research is recommended: to study the effect of different processing/treatments on these factors and how they affect the *in vitro* accessible iron.

ACKNOWLEDGEMENT

Table 1: Moisture, crude protein, crude fat content and citric acid of cowpeas, mung bean and kidney beans. Determined on duplicate samples (Mean ± S.D.)

Legume	Moisture (g/100g)	Crude protein (g/100g)	Crude fat (g/100g)	Citric acid (mg/100g)
Cowpea	9.7±0.3	21.7±0.1	2.4±0.2	121.7±9.8
Mungbean	10.0±0.5	21.9±0.2	2.3±0.2	70.8±5.7
Kidney bean (red V.)	9.9±0.2	18.8±0.1	1.7±0.1	184.0±14.8
Kidney bean (brown V.)	10.2±0.2	21.1±0.2	2.0±0.1	120.1±9.7
Kidney bean (reddish/brown V.)	10.7±0.1	21.4±0.3	1.8±0.2	205.2±16.6
Kidney bean (yellow V.)	10.5±0.1	20.3±0.5	2.9±0.1	107.3±8.7

Table 2: Phenolic groups and phytate content in cowpea, mung bean and kidney bean Determined on duplicate sample (Mean ± S.D.)

Legume	Antinutritional compounds				
	Total phenols[1]	Resorcinols[1]	Catechols[1]	Galloyls[2]	Phytates[3]
Cowpea	9.14±0.80	5.37±0.24	3.51±0.13	1.52±0.02	13.18±0.16
Mungbean	5.80±0.60	1.76±0.15	2.82±0.25	0.74±0.17	8.46±0.15
Kidney bean (red V.)	5.26±0.50	2.85±0.05	2.44±0.10	0.22±0.01	9.68±0.01
Kidney bean (brown V.)	5.83±0.35	2.89±0.15	3.18±0.22	0.35±0.05	11.31±0.24
Kidney bean (reddish/brown V.)	4.68±0.10	1.79±0.10	2.86±0.41	0.35±0.08	13.04±0.02
Kidney bean (yellow V.)	3.47±0.03	1.41±0.04	1.58±0.10	0.10±0.02	12.17±0.22

Table 3: Iron content and in-vitro iron accessibility (percentage in parenthesis) in cowpeas, mung beans and kidney beans. Determined on duplicate sample (Mean ± S.D.)

Legume	Total iron (mg/100g)	*In vitro* accessible iron (mg/100g)
Cowpea	4.74±0.04	0.53±0.01 (11.2)
Mungbean	3.58±0.06	0.45±0.01 (12.5)
Kidney bean (red V.)	5.79±0.03	0.82±0.02 (14.2)
Kidney bean (brown V.)	5.58±0.07	0.60±0.02 (10.7)
Kidney bean (reddish/brown V.)	7.55±0.08	1.04±0.03 (13.8)
Kidney bean (yellow V.)	5.52±0.09	0.71±0.01 (12.9)

1mg catechin equivalent per gram sample
2 mg tannic equivalent per gram sample
3 mg/g sample phytic acid

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