

Full Length Research Paper

## Effect of cooking on tannin and phytate content in different bean (*Phaseolus vulgaris*) varieties grown in Tanzania

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Phytates and tannins are present in varying proportions in legume based foods. Investigation on the effect of cooking on tannins and phytates was carried out on thirty eight raw and cooked bean (*Phaseolus vulgaris*) varieties. Tannins were assayed by vanillin-hydrochloric acid method, while phytates were determined by a method developed by Haugh and Lantzsch. There was a very high significant difference ( $P \leq 0.00001$ ) in the tannin concentrations between the raw and cooked bean samples. The mean tannin content for the raw and cooked samples was  $1.168 \pm 0.81$  and  $0.563 \pm 0.503\%$ CE, respectively. Reduction of tannins after cooking ranged from 20%CE in M'mafutala to 81%CE in GLP 2 with an average reduction of 56.3%CE. Equally, there was a very high significant difference ( $P \leq 0.0001$ ) in the phytate concentrations between the raw and cooked bean samples. The mean phytate content for the raw and cooked samples were  $0.0219 \pm 0.002$  and  $0.0122 \pm 0.003$   $\mu\text{g/mL}$ , respectively. Cooking reduced the anti-nutritional factors significantly ( $p \leq 0.0001$ ). The extent of anti-nutritional factors reduction varied between bean varieties. Cooking is therefore important for mineral absorption during digestion process in humans as it makes the minerals less bound and hence physiologically available.

**Key words:** Beans, phytates, tannins, minerals, raw, cooking, bioavailability.

### INTRODUCTION

The food that has been eaten and swallowed is mixed with saliva, gastric fluids and churned by peristaltic

movements of the stomach into a creamy fluid called the chyme. The chyme is an extremely complex matrix, in

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which the physico-chemical conditions change continuously with diet (Päivi et al., 2003). The absorption of mineral elements is a very complicated system and several components in foods can form soluble and insoluble complexes with these elements under intestinal conditions (Gibson et al., 2010). Legumes, cereals and other plant related foods, which form the base of diets for most African communities, contain phytate (*myo*-inositol hexaphosphate and other inositol phosphates), a known inhibitor of iron and zinc absorption (Hurrell et al., 1992; Lönnerdal, 2000; Afify et al., 2011). Normally encountered levels of phytates in cereals and legumes can reduce protein and amino acid digestibility by up to 10% (Gilani et al., 2012). Phytate-containing foods may be a strong contributing factor for poor iron and zinc status in population that consume these diets (Gibson et al., 2010). Common beans (*Phaseolus vulgaris*) are a staple food and the major source of iron for populations in Eastern Africa and Latin America but a major constraint to beans, is low iron absorption, attributed to inhibitory compounds (Petry et al., 2015). Several attempts could be done to reduce anti-nutritional effects of phytate such as soaking, drying, germination and drying. In a study conducted in Egypt to reduce phytate content, the phytate was significantly reduced from 23.59 to 32.40% and 24.92 to 35.27% for soaking and germination treatments, respectively (Afify et al., 2011). However, absorption of mineral elements from diets is likely to be higher with an enhancer when compared with low-bioavailability diets (Collings et al., 2013). Phytic acid inhibits iron absorption in a dose-dependent manner above a molar ratio of phytate to iron of 1:7 (Hurrell, 1992). However, ascorbic acid counteracts the effect of phytate when ascorbic acid:iron ratio exceeds 4:1 (Zijp et al., 2010). In legume grains, phytate is located in the protein bodies in the endosperm and occurs as a mineral complex, which is insoluble at physiological pH of the intestine (Sandberg, 2002). In a study in Malawi, a high intake of phytate was correlated with poor iron and zinc status in pre-school children (Gibson et al., 2010).

Similarly, tannins (polyphenols) are most commonly found in dicotyledons, particularly in Leguminosae (Salunkhe et al. 1990). With regard to the legume grains, tannins are found in dry beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum*), chickpeas (*Cicer arietinum* L.), faba beans (syn. broad bean, field bean; *Vicia faba* L.), cowpeas (*Vigna unguiculata* L.) and lentils (*Lens culinaris* L.). In most grain legumes, tannins are present as condensed tannins (Salunkhe et al., 1990). According to Hurrell et al. (1999) and Yuwei (2013), certain polyphenols are able to form complexes with iron, which makes the complex-bound iron unavailable for absorption. Brune et al. (1989) reported that the amount of iron binding phenolic galloyl groups in foods roughly corresponds to the degree of inhibition of iron absorption. However, Hurrell et al. (1999) concluded that all major types of food polyphenols can strongly inhibit dietary non-

heme iron absorption. On the other hand, literature reveals that plant tannins play a protective role in the defence of plants against environmental influences. Increased concentrations of tannins therefore have been found in plants under environmental stress as compared to similar plants without environmental stress (Islam et al., 2002; Yuwei, 2013).

Most studies have investigated the content and effect on mineral and protein absorption of tannins and phytates in foods. However, few studies have explored the effect of cooking on the levels of these anti-nutritional factors. The aim of the study therefore was to investigate whether conventional cooking had an effect on reducing the levels of tannins and phytates in the *P. vulgaris* beans which is among the major protein and mineral source in the diet of most African communities.

## MATERIALS AND METHODS

### Bean samples

A total of 38 *P. vulgaris* bean seed varieties were obtained from University of Nairobi, Kenya and multiplied at Sokoine University of Agriculture, Morogoro Tanzania to obtain enough beans to be subjected to cooking and laboratory analysis for tannins and phytates. Tannin and phytate content were determined on raw and cooked bean grain samples. The analyses were done in the Department of Food Technology, Nutrition and Consumer Sciences, in collaboration with the Department of Animal Science and Production laboratories.

### Preparation of raw and cooked bean grain samples

For each variety, two batches of bean grain samples were prepared. One batch was ground in its raw state and bean flour was obtained. The second batch was boiled in water as it is often done in homesteads, until cooked. The samples were mashed into thick paste and put in oven maintained at 105°C for 24 h. Tannin and phytate analysis was carried out on the raw and cooked samples.

### Determination of tannin content

A quantitative value of tannin was assayed by vanillin-hydrochloric acid method. For each bean variety, about 0.25 g of milled sample was weighed and placed into Erlenmeyer flask. 10 mL of 4% HCl in methanol was pipetted in a flask. The flask was covered with para-film and shaken for 20 min on a wrist action shaker. The extract was transferred into centrifuge tubes and centrifuged for 10 min at 4500 rev/min. The supernatant aliquot was transferred into 25 mL flask and the residue from centrifuge tube was rinsed using 5 mL of 1% HCl in methanol, covered with para-film and shaken for another 20 min. The residue mixture was centrifuged again for 10 min and the aliquot were combined with the first extract.

A set of catechin standard solutions were prepared and added into test tubes with 1 mL absolute ethanol. Slowly, 5 mL of vanillin-HCl reagent was added in each sample and 1 mL sample extract. A blank sample with 5 mL of 4% HCl in absolute methanol was prepared. The absorbance of standard solutions, sample extracts and sample blanks were read on a spectrophotometer set at 500 nm exactly 20 min after adding vanillin-HCl reagent to the standard solutions and sample extracts. A standard curve absorbance (*y*) against catechin concentration (*x*) was prepared from the catechin

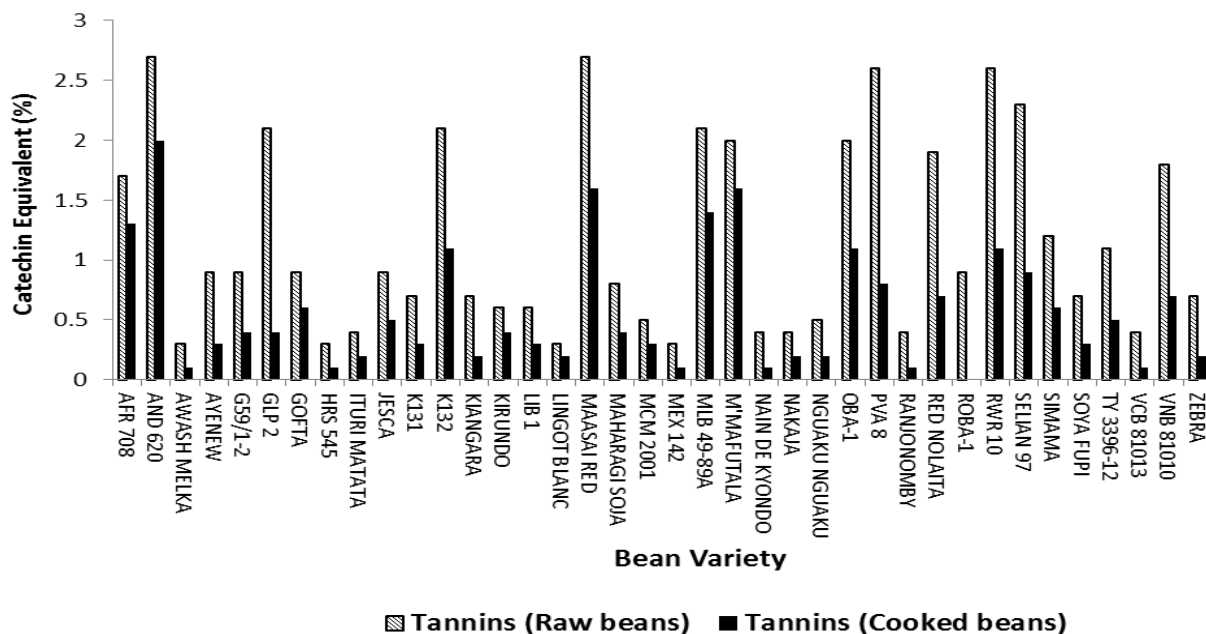


Figure 1. Effect of cooking on tannin levels in raw and cooked beans.

standard solution readings and the slope was computed and hence tannin concentration was determined from the equation of the curve.

$$CE(\%) = \frac{CC \times VM}{VE \times Wt}$$

Where CE = catechin equivalent; CC = catechin concentration (mg mL<sup>-1</sup>); VM = volume made up (mL); VE = volume of extracts and Wt = weight of sample.

#### Determination of phytate content

Phytate was determined by a sensitive method developed by Haugh and Lantzsch (1983). About 1 g of bean samples was weighed and phytate was extracted with 10 mL of 0.2 N HCl. During the extraction process, the mixture was stirred for 30 min by using a magnetic stirrer. To a 0.5 mL, 1 mL of ammonium iron III sulphate in HCl (0.2 g ammonium iron (III) sulphate 12H<sub>2</sub>O in 100 mL of 2N HCl and made up to 1 L) was added in the test tube and boiled for 30 min in a boiling water bath and then cooled to room temperature in ice water. The contents of the tube were mixed and centrifuged for 30 min at 3000 rev/min. 1 mL of the supernatant was transferred to another tube and 1.5 mL of 2,2'-bipyridine solution (10 g of 2,2'-bipyridine with 10 mL thioglycolic acid in 1000 mL water) was added. Absorbance of the solution was determined at 519 nm wavelength against distilled water. A standard curve was prepared using phytate-phosphorus at concentration between 3 and 30 µg/mL treated the same way but without the sample. All determinations were done in triplicate.

#### Statistical analysis

Data for all the determinations were analysed using excel office 2010 and statistical product and service solutions (SPSS

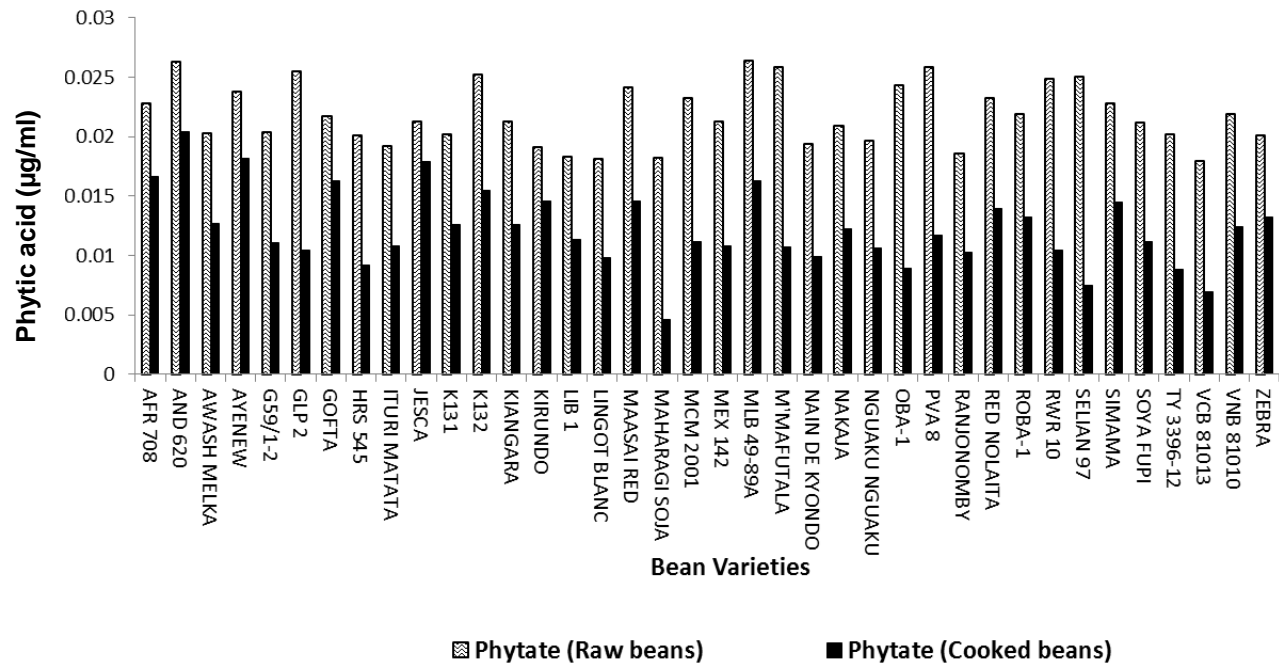
version 16) computer software. Students t-test was used to test for significance difference between the treatment means for each variety (Fresh and cooked), while one way analysis of variance was used to test for significance differences among the varieties. Mean separation were done by Duncan multiple range test at P<0.05.

## RESULTS

### Effect of cooking on tannins and phytates contents of *P. vulgaris* bean varieties

The tannin and phytate levels in the *P. vulgaris* bean varieties are presented in Figures 1 and 2. A paired t-test analysis showed a very significant difference ( $P \leq 0.00001$ ) in the tannin concentrations between the raw and cooked bean samples. The mean tannin content for the raw and cooked samples were  $1.168 \pm 0.81$  and  $0.563 \pm 0.503\%$ CE, respectively with  $0.457-0.753\%$ CE 95% confidence interval of the mean difference. The minimum and maximum tannin values for the raw and cooked samples were 0.3 to 2.7 and 0.0 to 2.0%CE, respectively. The raw and cooked bean varieties with highest tannin concentrations were Maasai Red and (2.7 and 1.6%CE) and AND 620 (2.7 and 2.0%CE) while the lowest was Lingot Blanc (0.3 and 0.2%CE) and MEX 142 (0.2 and 0.1%CE), respectively (Figure 1).

Similarly, phytate concentration varied significantly between varieties (Figure 2). There was a very significant difference ( $P = 0.0001$ ) in the phytate concentrations between the raw and cooked bean samples. The mean phytate content for the raw and cooked samples were  $0.0219 \pm 0.002$  and  $0.0122 \pm 0.003$  µg/mL, respectively with



**Figure 2.** Effect of cooking on phytate levels in raw and cooked beans.

0.0086 to 0.0107 µg/mL 95% confidence interval of the mean difference. The minimum and maximum phytate values for the raw and cooked samples were 0.021 to 0.0227 and 0.0111 to 0.0133 µg/mL, respectively. The raw bean varieties with highest phytate concentrations were MLB 49-89A (0.026 µg/mL) and AND 620 (0.0263 µg/mL), while the lowest was VCB 8101 (0.018 µg/mL) and Maharagisoja (0.0182 µg/mL).

#### Percentage reduction of tannins and phytates after cooking of bean samples

A one way analysis of variance showed a very significant reduction ( $P=0.0001$ ) in tannins from raw to cooked bean samples. Tannin reduction ranged from 20% in M'mafutala to 81% in GLP 2 with an average reduction of  $56.3 \pm 15.5\%$  of all the samples tested with 0.48 to 58.76% confidence interval of the mean (Table 1). Equally, there was a very significant reduction ( $P=0.0001$ ) in phytate from raw to cooked bean samples. Phytate reduction ranged from 16% in Jesca to about 75% in Maharagisoja with an average reduction of  $44.2 \pm 13.3\%$  of all the samples tested with 39.7 to 48.57% 95% confidence interval of the mean (Table 1).

#### DISCUSSION

The results revealed that cooking has an effect of reducing the anti-nutritional factors of *P. vulgaris* bean,

although the extent of reduction varies from one bean variety to the other. With regard to mineral absorption, cooking is therefore advantageous as an amount of minerals and proteins will be less bound and hence become physiologically available. A recent review has revealed that cooking improves nutritional quality of beans as well as other vegetables which contain high amounts of anti-nutritional factors in raw state (Fabbri and Crosby, 2016). In some studies, cooking decreased significantly ( $p \leq 0.05$ ) polyphenol content inherent in the karkade seeds, but other processing methods studied did not (El Gasim et al., 2008). In another study, cooking resulted in a high reduction of phytate and oxalates on green leafy vegetables (Ilelaboye et al., 2013). Heat degradation, leaching out effects, change in chemical reactivity and formation of insoluble complexes might be the factors that resulted in the significant reduction of these anti-nutrients by cooking (Alonso et al., 2000; Yagoub et al., 2004; Wu et al., 2016). Other studies however, gave mixed results, the phytic acid content of karkade seeds (888.33 mg/100 g) and sorghum genotypes were unaffected by soaking, sprouting and cooking (Alonso et al., 2000; Wu et al., 2016). Additionally, it was observed that most of the bean varieties, which were black and red coloured such as GLP 2, K132, PVA8 and RWR 10 were the ones with relatively highest tannins as compared to the white, yellow and light brown coloured such as Awashmelka, HRS 545 and Ranjonby; although colour was not a factor investigated in this study.

According to Manan et al. (1987), the effects of a

**Table 1.** Percentage tannin and phytate reduction of cooked bean samples.

Bean variety	Tannin reduction (%)	Phytate reduction (%)	Bean variety	Tannin reduction (%)	Phytate reduction (%)
MAHARAGI SOJA	50.0	74.7	VNB 81010	61.1	43.4
SELIAN 97	60.9	70.1	NAKAJA	50.0	41.6
OBA-1	45.0	63.4	KIANGARA	71.4	40.8
VCB 81013	75.0	61.7	RED NOLAITA	63.2	40.3
GLP 2	81.0	59.2	ROBA-1	55.6	39.7
M'MAFUTALA	20.0	58.7	MAASAI RED	40.7	39.7
RWR 10	57.7	58.2	K132	47.6	38.5
TY 3396-12	54.5	56.4	MLB 49-89A	33.3	38.3
PVA 8	69.2	54.8	LIB 1	50.0	38.3
HRS 545	66.7	54.2	K131	57.1	37.6
MCM 2001	40.0	51.9	AWASH MELKA	66.7	37.4
MEX 142	66.7	49.3	SIMAMA	50.0	36.4
NAIN DE KYONDO	75.0	49.0	ZEBRA	71.4	34.3
SOYA FUPI	57.1	47.2	AFR 708	23.5	27.2
NGUAKU NGUAKU	60.0	46.2	GOFTA	33.3	24.9
LINGOT BLANC	33.3	45.9	KIRUNDO	33.3	23.6
G59/1-2	55.6	45.6	AYENEW	66.7	23.5
RANJONOMBY	75.0	44.6	AND 620	25.9	22.4
ITURI MATATA	50.0	43.8	JESCA	44.4	16.0

normal cooking process on the phytic acid and the nutritional quality of three varieties of Pakistani peas and lentils, which involved steeping, followed by boiling of the seeds, resulted in a reduction of considerable amounts of phytic acid in peas and lentils by 82 and 76%. The study revealed that boiling caused the highest reduction in tannins followed by autoclaving and microwave cooking. Autoclaving and fermentation were the most effective in reducing phytic acid content. However, there was no apparent relationship observed between the loss of phytates from the seeds and the improvement of nutritional quality. A study in Nigeria by Nzewi and Cemauck (2011) reported a significant reduction of phytate from  $1.54 \pm 0.04$  to  $0.18 \pm 0.11$  mg/g, representing 88% reduction in boiled asparagus beans. Similarly, tannins were reduced from  $3.72 \pm 0.12$  to  $1.88 \pm 0.08$  mg/g or 49% reduction. Iorgyer et al. (2009) studied the effect of boiling on pigeon pea seeds and reported no significant effect on phytates but cooking resulted in 47.06% destruction of tannins in pigeon peas.

In another study, Khattab and Arntfield (2009) observed that the residual content of antinutritional factors of cowpeas, peas and kidney bean seeds after soaking, boiling, roasting, microwave cooking, autoclaving, fermentation and micronization resulted in significant decreases in tannins, phytic acid, trypsin inhibitor activity and oligosaccharides as compared to the raw seeds. Similarly, Vijayakumari et al. (1996) revealed that cooking for 3 h resulted in significant reduction of phytic acid, raffinose, stachyose and verbascose in both germplasms

of the Indian tribal pulse, *Mucuna monosperma*, while autoclaving was more effective in eliminating the contents of phytic acid in the germplasms.

In several single-meal studies, phytate has been shown to significantly inhibit iron and zinc absorption, but few studies have explored the effects of long-term modification of phytate intake (Yuwei, 2013). Lind et al. (2003) assessed the longitudinal effects of the extensive reduction of the amount of phytate in infant cereals, a common complementary food, on micronutrient status. Despite a reduction in the daily phytate intake from infant cereals of 77%, they found no greater effect on haemoglobin, serum ferritin, or serum zinc than that with commercial complementary cereals, which were richer in phytate. Feeding infant formula, with its lower iron content but presumably higher bioavailability, resulted in a significantly lower haemoglobin and higher prevalence of anaemia than did feeding phytate-reduced infant cereal. Iron-fortified infant cereals are commonly used in complementary diets (Mamiro et al., 2004). Other antinutritional compounds such as phytohaemagglutinins, which are alectin present in many varieties of common bean, especially red kidney and faba beans can be deactivated by cooking beans at 100°C (212°F) for ten minutes (Yuwei, 2013).

## Conclusion

Conventional cooking has an effect on reducing the

tannins and phytates present in *P. vulgaris* beans. This is beneficial in that some amount of minerals will not be bound and hence become physiologically available for metabolic activities. This is advantageous for the vegetarians and most African households with respect to mineral intake due to the fact that beans are an important food item consumed to a large extent and mostly on daily basis.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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