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EFFECT OF GERMINATION AND ROASTING ON THE PROXIMATE, MINERAL AND ANTI-NUTRITIONAL FACTORS IN FINGER MILLET (*Eleucine coracana*), COWPEAS (*Vigna unguiculata*) AND ORANGE MAIZE (*Zea mays*)

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ABSTRACT

Finger millet (*Eleucine coracana*), cowpea (Vigna unguiculata), and bio-fortified vitamin A "orange" maize (Zea mays) are three nutrient dense crops currently being promoted in Zimbabwe. The effect on nutrient content of processing these specific crop varieties has not been investigated. Therefore, this study was designed to determine the effects of germination and roasting on the proximate, mineral, and anti-nutritional factors in finger millet, cowpeas and orange maize. Finger millet grains were germinated for 48hrs, cowpeas and orange maize for 24hrs, at room temperature (20-23^{o}C). Both raw and processed samples were dried and milled into flour for the determination of proximate and mineral and anti-nutritional composition. Protein content of finger millet increased significantly after processing from 6.53±0.25 mg/100 g to 11.27±0.15 mg/100 g in germinated finger millet flour ($P<0.05$). Germination of finger millet resulted in significantly increased minerals (mg/100 g); calcium from 345.53±0.55 to 352.63±0.21, zinc from 3.59±0.15 to 8.71±0.01, sodium from 49.89±0.16 to 57.78±1.20 and iron content from 3.75±0.05 to 4.52±0.01 whilst magnesium and potassium decreased significantly from 198.09±0.07 to 69.08±0.06 and 487.08±0.03 to 144.78±0.27 respectively. Processing of cowpeas resulted in slight but significant increase in protein content (20.47±0.21 to 28.50±0.10), increased calcium (138.18±0.12 to 148.18±0.12 mg/100 g), magnesium (14.23±2.00 to 19.18±0.31 mg/100 g), potassium (232±4.00 to 443.41±0.02 mg/100 g) and iron (4.85±0.03 to 4.86±0.04 mg/100 g). Conversely zinc and sodium decreased from 4.5±0.30 to 2.9±0.10 mg/100 g and 31.85±0.03 to 11.64±0.02 mg/100 g, respectively. Notably for orange maize, protein content did not change from 10.06±0.04 to 10.04±0.04 g/100 g before and after processing. Calcium increased from 47.02±2.82 to 57.99±8.85 (mg/100 g), magnesium from 90.91±0.11 to 108.30±0.53 (mg/100 g), potassium from 2.13±0.04 to 4.33±0.25 (mg/100 g), sodium from 0.50±0.02 to 0.70±0.02 (mg/100 g) and iron from 0.50±0.02 to 1.25±0.05 (mg/100 g). Zinc decreased from 6.2±0.2 to 3.53±0.55 (mg/100 g). Tannins, oxalates and phytates decreased significantly after processing of all three crops. Results showed that germination and roasting increased the nutritional profile and decreased anti-nutrient content in finger millet, cowpeas and orange maize. Therefore, it is important to consider germinating and roasting these grains during processing to increase the nutritional potential of the end food product. Further studies are required to investigate the decrease in some nutrients after germination and roasting and possibly establish optimum processing parameters for improved nutrient profile of these food crops.

Key words: Traditional grains, millet, orange maize, biofortification, germination, roasting, nutrients, anti-nutrients

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INTRODUCTION

Finger millet (FM) (*Eleucine coracana*) is widely grown in Zimbabwe with variations in colour (brown, white and light brown cultivars). The grain is also characterized by a high concentration of carbohydrates, dietary fibre, phytochemicals and essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine); presence of essential minerals; as well as a gluten-free status. Finger millet grain is extremely neglected and widely underutilized [1]. It is a good source of calcium, iron, phosphorus, zinc, potassium, other minerals and fibre [2]. It is also an important staple food because of its excellent storage properties, and nutritive value, resistance to disease and tolerance to soil moisture stress [3]. Its nutritive value is comparable to rice with regard to protein (6-8%) and fat (1-2%) and is superior to rice and wheat with respect to mineral, fibre and micronutrient contents [4].

Cowpeas (*Vigna unguiculata*) is a legume consumed as a source of high-quality plant protein in many parts of the world. High protein and carbohydrate contents with the relatively low fat content and complementary amino acid pattern to that of cereal grains make cowpea an important nutritional food in the human diet [6]. Cowpea seeds are rich in protein (18–25%) [7] and the fat content in cowpea is 1.4%-2.7% [8], with about 6% crude fibre [6]. This makes cowpeas a very key ingredient in the production of complementary foods. Amino acids derived from cowpea are an effective complement to those obtained from cereals. This is because cowpeas have more lysine, threonine and tryptophan, while cereals are rich in methionine, which is deficient in cowpeas [6]. Cowpea protein isolates have good functional properties, including foaming and emulsifying activities in addition to solubility [9]. Cowpeas possess significant anti-inflammatory effects, mediated by specific phenolic profiles and antioxidant activity.

Orange maize is maize bio-fortified with carotenoids which are precursors to vitamin A. This vitamin has important functions in vision and immunity. Approximately one third of children under the age of five are at risk for Vitamin A deficiency [10]. This is the leading cause of preventable childhood blindness and can be combated through the consumption of vitamin A rich foods such as orange maize. Due to the emerging threat of climate change and persistent high prevalence of malnutrition, food product development has shifted focus to involve ingredients that are climate smart and highly nutritious varieties such as finger millet, cowpeas and biofortified orange maize. These two cereals and legume are commonly used in African cuisine after some processing at household level. However, the effects of the processing on nutrition and non-nutrition components is

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not fully documented. Though there is scientific evidence that processes like germination and roasting improve mineral content of grains and reduce anti nutrients, there is limited evidence concerning the combined effect of germination and roasting on new varieties of orange biofortified maize, cowpeas and finger millet.

Germination is a common household technique carried out at low cost without the use of any sophisticated and expensive equipment for the biochemical enrichment of grains involving the transition of a seed from a dormant state to vital active state [2]. Germination reduces anti-nutrients namely tannins and phytates [5], thereby improving nutritional and functional properties of millet. The mousy odour of damp millet is also eliminated [11]. In addition, germination maximizes the optimum level of absorbable nutrients [12]. Germination has been reported to improve the nutritional quality of seeds by increasing the contents and availability of essential nutrients and lowering the levels of anti-nutrients [13]. During roasting of finger millet, the antinutritional or toxic effects of compounds such as saponins, alkaloids, glycosides, goitrogenic agents, trypsin inhibitor and hemagglutinin are removed. Roasting of finger millet, improves the nutritional quality and increases the shelf-life of the roasted grains [1]. Milled finger millet is rich in dietary fibre and micronutrients [14]. In African households, grains are normally germinated and roasted before further processing into food products. This study was designed to assess the effect of germination and roasting on the proximate, mineral and anti-nutrients in finger millet, cowpeas and orange maize, as part of a bigger project to formulate an infant instant porridge. The expected outcome was to reduce the antinutritional components in finger millet and cowpeas such as tannins and oxalates, and to produce a nutrient dense composite flour that can be used to produce an instant porridge. Current complementary foods are based on wheat or maize which unless fortified are low in micronutrient content hence exacerbating the problem of malnutrition in developing countries like Zimbabwe. Further, in the face of climate change, novel foods from drought resistant varieties need to be promoted for sustainable food systems.

MATERIALS AND METHODS

Grain processing

The finger millet seeds were purchased from Mashonaland West province, Karoi town (16°48'36"S 29°42'00"E), orange maize from Manicaland province, Mutasa district (18°35'S 32°45'E) and the cowpeas were obtained from Manicaland, Buhera district (19°19' 57.00"S, 31° 26' 6.00"E), Zimbabwe. The grains and legume were taken for analysis at the Department of Nutrition Dietetics and Food

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Science laboratory, Faculty of Science, University of Zimbabwe, Zimbabwe. Stones and unwanted materials were handpicked. The samples were washed, soaked for 3hrs and germinated for 48hrs (finger millet) and 24hrs (orange maize and cowpeas) according to household standard practice, at room temperature (20-23^{o}C). Germination was done in sacks at room temperature, and water was sprinkled regularly at 3hr intervals. After germination, the samples were sun dried till the moisture content was 10-12%. The germinated grains were then roasted in an oven at a temperature of 60^{o}C for 10-15minutes. The samples were later ground, filtered with a sieve of 2 mm mesh to fine powder, then kept in a polythene bag for analysis.

Proximate analysis

The proximate analysis of the raw and germinated-roasted samples of orange maize, cowpeas and finger millet for moisture, crude protein, crude fibre, ash, carbohydrate and fat were carried out in triplicates according to the method of Association of Official Analytical Chemists (AOAC, 1995). Nitrogen was determined by the micro Kjeldahl method as described by Pearson [34] and the percentage nitrogen was converted to crude protein by multiplying by 6.25. The carbohydrate content was determined by the difference method. That is total carbohydrates was the residual weight after subtracting total water, total protein, total ash and fat.

Mineral analysis

For mineral analysis, samples were digested by nitric acid and the respective mineral concentration determined using computer controlled Atomic Absorption Spectrometer (AAS Model AA-6701F), Shimadzu, Japan. All determinations were done in triplicate and mineral content was reported in mg/100 g sample. The minerals reported here are zinc, iron, calcium, magnesium, potassium, sodium and iron.

Anti-nutrient analysis

1. Phytates

Phytates were determined by the method by Young [15]. Powdered samples of finger millet, cowpeas and orange maize were separately extracted in 2.4% HCl for 3 hrs (0.1g/5 ml). The supernatant (0.5 ml) was mixed with 0.5 ml 2.4% HCl and 0.2 ml $FeCl_3$ (0.00145gFe/ml 1N HCl), then boiled for 15 min and cooled thereafter. To 1 ml of supernatant, we added 0.25ml N/2 HCl add 0.5ml 10% KCNS and 2.5 ml N/6 HCl. A blood red colour developed whose absorbance was read at 540nm using a spectrophotometer (Biobase BK-D560, China). The content of phytate was expressed as mg/100 g.

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2. Tannins

Tannins were determined by the method by Rybak-Chmielewska [16]. One gram of each finger millet, orange maize and cowpeas sample was separately weighed into a beaker. Each sample was soaked with a solvent mixture (80 ml of acetone and 20 ml of glacial acetic acid) for 5 hrs to extract tannins. The samples were filtered through a double layer filter paper to obtain the filtrates which were stored for further use. A standard solution of tannic acid was prepared ranging from 10 ppm to 30 ppm. The absorbances of the standard solutions as well as that of the filtrates were read at 500 nm on a Spectrophotometer.

3. Oxalates determination

Oxalate determination was as previously described by Adeniyi *et al.* [17]. Two grams of the sample were digested with 10 ml 6 M HCl for one hour and made up to 250 ml in a volumetric flask. The pH of the filtrate was adjusted with concentrated NH_4OH solution until the colour of solution changed from salmon pink colour to a faint yellow colour. Thereafter, the filtrate was treated with 10 ml of 5% $CaCl_2$ solution to precipitate the insoluble oxalate. The suspension was centrifuged at 2500 rpm, after which the supernatant was decanted and precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4. The total filtrate resulting from the dissolution in H_2SO_4 was made up to 300 ml. An aliquot of 125 ml of the filtrate was heated until near boiling point and then titrated against 0.05 M of the standardized $KMnO_4$ solution to a faint pink colour which persisted for about 30 seconds after which the burette reading was taken. The oxalate content was evaluated from the titre value.

Statistical analysis

Data was entered into excel and analysed using Statistical Package for the Social Sciences (SPSS). Paired t-test [18] was used to test for significant differences between means (before processing and after processing). Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Finger Millet

Proximate analysis of raw, germinated and roasted finger millet was done and results shown in Table 1. It was found that the moisture content of raw finger millet flour and germinated then roasted finger millet flour was 12.05±0.02 and 14.70±0.15 g/100 g, respectively. This increase in moisture was significant ($P<0.05$). Total ash content was 2.42±0.03 and 2.4± 0.35g/100 g for raw finger millet flour and germinated then roasted finger millet flour respectively. Fat content

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was 1.23±0.03 and 1.52±0.04 g/100 g for raw finger millet flour and germinated then roasted finger millet flour respectively. This difference was statistically significant (P>0.05). The germination process slightly increased the fat content of the finger millet. The crude fibre content of the germinated sample was lower 5.84±0.02 g/100 g compared the raw finger millet flour which was 18.28±0.02 g/100 g. This decrease in fibre content could be due to the fact that during germination, sugar in the seed is usually used up leaving only the small fibrous seed [19]. Raw finger millet flour had a protein content of 6.53±0.25 g/100 g and the germinated then roasted finger millet flour had a protein content of 11.27±0.15 g/100 g. The germination process increased significantly the protein content and this could be due to mobilization of stored nitrogen to produce nutritious and good quality protein required for the development of young plant [20]. The increase in protein content of the germinated millet may also be attributed to the formation of enzymes or the internal changes following degradation of other constituents [21]. Carbohydrate content for raw finger millet flour was 60.23±0.02 g/100 g. After germination and roasting, this increased significantly to 64.24±0.41 g/100g. Since carbohydrate content of plant food is calculated by difference; decrease in crude fibre and ash content of the finger millet after roasting will ultimately affect the value of carbohydrate content, hence the observed increase [22]. Further, the α-amylase breaks the complex carbohydrates into simple sugars which are utilized in growing seeds in the first phase of germination [23].

Cowpeas and orange maize

The effect of germination and roasting on cowpeas and orange maize are shown in Table 1. The moisture content of cowpea before and after germination with roasting was 10.70±0.10 and 5.97±0.21 g/100 g, respectively. The decrease in moisture content could be due to the free moisture that evaporates during roasting. The moisture content of orange maize before and after germination with roasting was 8.84±0.05 and 9.65±0.05 g/100 g respectively. For orange maize, the moisture content significantly increased after germination. After entry of water in the seed coat, seed swelling starts and initiates germination. This extra moisture contributes to the extra water content. Non-significant increase in the ash content was observed after germinating and roasting of cowpeas from 3.77±0.15 to 4.10±0.10. Increase in ash content may be apparent due to loss of starch [24].

There was a significant increase in protein content after germination and roasting in cowpeas from 20.47±0.21 to 28.50±0.1 g/100 g but no difference for orange maize (10.06±0.04 and 10.04±0.04 g/100 g respectively). Uppal and Bains [25] observed crude protein increase from 8 to 11% after germination in legumes. Apparent increase in protein content may be attributed to loss in dry matter,

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particularly carbohydrates through respiration during germination [25]. Longer germinating time would mean a greater loss in dry weight and more increase in crude protein content.

The fat content before and after germination/roasting decreased as follows; for orange maize it was 4.72±0.01 g/100 g and it decreased to 3.20±0.02 g/100 g. On the whole, it is reported that degradation of reserve nutrients (lipids and carbohydrates) during germination is a process whose essential purpose is required to provide the energy for protein synthesis in plant growth [26]. The observed decrease in the fat contents of the germinated seeds might be due to the increased activities of the lipolytic enzymes during germination [27]. For cowpeas, the fat content increased from 1.92±0.26 g/100 g to 3.19±0.03 g/100 g. This is associated with heat-induced break down of the bonds that exist between the fat and matrix of the cowpeas, resulting in efficient release/mobilisation of the oil reserve after roasting. The decreased carbohydrate levels of the germinated and roasted cowpeas might be due to an increase in α-amylase activity, which breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination [27].

Mineral analysis

The mineral analysis of the grains and legumes are shown in Table 2. Germination is expected to increase the mineral content due to an increase in phytase enzyme activity during germination. Phytase enzyme hydrolyzes the bond between the phytic acid and trapped minerals hence the minerals become free, increasing their availability [28]. Likewise, the calcium content of finger miller before and after germination with roasting was 345.53± 0.55 mg/100 g and 352.63 ± 0.21 mg/100 g respectively. That of orange maize increased from 47.02±2.82 to 57.99±8.85 mg/100g and cowpeas from 138.18 ±0.12 to 148.18 ± 0.12 mg/100 g. Decreases of oxalic acid during germination correspondingly increases calcium content in finger millet because oxalic acid is known to interfere with calcium absorption [29]. The increase in calcium in orange maize might be due to the loss of organic dry matter from the grains during germination and this increases in the percentage of calcium in the grains [30]. The zinc content for finger millet increased from 3.59 ± 0.15 mg/100 g to 8.71 ± 0.01 mg/100 g, whilst for orange maize the content decreased from 6.2±0.20 mg/100 g to 3.53±0.55 mg/100 g and lastly for cowpeas from 4.5±0.30 mg/100 g to 2.9±0.10 mg/100 g. The slightly lower zinc content observed in processed cowpeas may be a result of soaking, which allows zinc to leach into the water, as observed previously by Fasreen *et al.* [31]. However, cowpeas after processing can still provide a substantial amount of zinc in children's diets, especially considering that the daily recommended intake for children aged

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1–3 years is 3 mg/day. There were variations when it came to the other minerals magnesium, potassium and sodium. The levels of sodium in cowpeas decreased significantly after processing possibly due to leaching but increased for millet (49.89±0.16 to 57.78± 1.20 mg/100 g) and slightly for orange maize (0.50±0.02 and 0.70±0.02 mg/100 g). Magnesium and potassium levels decreased significantly in finger millet but both minerals increased in orange maize and cowpeas. These are minerals required in minute quantities in our diets. Even in their decreased state after processing, they can adequately fulfil the recommended dietary allowance (RDA) for children under 5 years of age. Iron is an important micronutrient that is lacking in most diets in developing countries. These diets are high in cereal based foods hence legumes become the second most important source of iron after meat products. The levels of iron increased after germination with roasting in both grains and legume analysed from 4.85 ± 0.03 to 4.86 ± 0.04 mg/100 g in cowpeas, 3.75± 0.05 to 4.52± 0.01 mg/100 g finger millet and 0.50±0.02 to 1.25±0.05 mg/100 g orange maize. Germination is therefore an important processing step that can improve iron content, hence, improve dietary iron intake in cereal based diets.

Anti-nutrients analysis

The results for anti-nutrient analysis is shown in Table 3. Phytic acid significantly decreased from 899.93±10.29 mg/100 g to 346.21±2.00 mg/100 g in finger millet due to increased phytase activity during germination, which hydrolyses the phytic acid to phosphate and myoinositol phytates [32]. This breakdown of phytate during germination is attributed to the increased activity of the endogenous phytase (enzyme activity). Since phytic acid has been considered to be one of the factors responsible for reducing mineral bioavailability, its reduction during germination may have enhanced the nutritional quality (with respect to mineral bioavailability) of finger millet [33]. Processing methods such as soaking, fermentation, and germination have been earlier reported to reduce phytate content and oxalates for some seeds [33]. In orange maize and cowpeas, phytate levels significantly decreased after germination with roasting due to the same reason of mobilisation of the enzyme phytase.

The tannin levels were reduced significantly in both grains and legumes (874.23±5.48 to 360.45±0.04 mg/100 g finger millet, 28.00±4.00 to 20.00±2.00 mg/100 g orange maize, 390.90±0.79 to 149.34±0.34 mg/100 g cowpeas). The decrease in tannin content during germination has been explained as leaching of tannin from the sprouting mass and decreased activity of polyphenol oxidase and other metabolic enzymes [33]. Tannins are also inhibitors of nutrient absorption therefore a reduction in content is advantageous in diets high in plant-based foods.

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Oxalate content is of nutritional importance because of interference with calcium bioavailability [11]. This was also reduced significantly possibly due to leaching and roasting effects [33]. This decrease was most apparent in orange maize (590.00±5.00 to 42.50±1.00 mg/100 g and cowpeas (339.79±0.27 to 65.88±0.20 mg/100 g) but much less in finger millet 48.66±0.05 to 33.54±0.05 mg/100 g.

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

Finger millet, cowpeas and orange maize are rich sources of nutrients. However, processing can differentially affect nutritional value. Germination brought about an appreciable increase in protein, fibre, and some minerals in finger millet, cowpeas and orange maize and a significant decrease in anti-nutrients. The combination of these grains and legume after germination with roasting will likely compliment individual nutritional profiles, thus making them ideal ingredients in the making of food products for vulnerable groups. Recommendations for further studies in vitamins in finger millet, cowpeas and orange maize are also needed.

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Table 1: Proximate analysis of raw and germinated and roasted finger millet, orange maize and cowpeas (g/100 g)*

	Raw finger millet	Germinated and roasted finger millet	P value	Raw orange maize	Germinated and roasted orange maize	P value	Raw cowpeas	Germinated and roasted cowpeas	P value
Moisture	12.05±0.02	14.07±0.15	0.001	8.84±0.05	9.65±0.05	0.001	10.70±0.10	5.97±0.21	0.001
Protein	6.53±0.25	11.27±0.15	0.001	10.06±0.04	10.04±0.04	0.100	20.47±0.21	28.50±0.10	0.001
Crude fibre	18.28±0.02	5.84±0.02	0.001	1.41±0.02	1.20±0.02	0.001	6.01±0.04	5.95±0.03	0.188
Fat	1.23±0.03	1.52±0.04	0.015	4.72±0.01	3.20±0.02	0.001	1.92±0.26	3.19±0.03	0.001
Ash	2.42±0.03	2.40±0.35	0.923	2.65±0.04	2.14±0.04	0.001	3.77±0.15	4.10±0.10	0.109
Carbohydrates	60.23±0.02	64.24±0.41	0.003	72.31±0.04	73.77±0.05	0.001	57.13±0.96	52.29±0.39	0.004

*Paired T test used. Significance set at $p<0.05$

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Table 2: Mineral analysis of finger millet, orange maize and cowpeas (mg/100 g)

	Raw finger millet	Germinated and roasted finger millet	P value	Raw orange maize	Germinated and roasted orange maize	P value	Raw cowpeas	Germinated and roasted cowpeas	P value
Calcium	345.53±0.55	352.63±0.21	0.003	47.02±2.82	57.99±8.85	0.088	138.18±0.12	148.18±0.12	0.001
Zinc	3.59±0.15	8.71±0.01	0.001	6.20±0.20	3.53±0.55	0.025	4.5±0.30	2.9±0.10	0.005
Magnesium	198.09±0.07	69.08±0.06	0.001	90.91±0.11	108.30±0.53	0.059	14.23±2.00	19.18±0.31	0.064
Potassium	487.08±0.03	144.78±0.27	0.001	2.13±0.04	4.33±0.25	0.005	232.00±4.00	443.41±0.02	0.001
Sodium	49.89±0.16	57.78±1.20	0.007	0.50±0.02	0.70±0.02	0.001	31.85±0.03	11.64±0.02	0.001
Iron	3.75±0.05	4.52±0.01	0.001	0.50±0.02	1.25±0.05	0.003	4.85±0.03	4.86±0.04	0.057

*Paired T test used. Significance set at p<0.05

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Table 3: Anti-nutrients analysis of finger millet, cowpeas and orange maize (mg/100 g)

Anti-nutrients	Raw finger millet	Germinated and roasted finger millet	P value	Raw orange maize	Germinated and roasted orange maize	P value	Raw cowpeas	Germinated and roasted cowpeas	P value
Tannins	874.23±5.48	360.45±0.04	0.001	28.00±4.00	20.00±2.00	0.120	390.90±0.79	149.34±0.34	0.001
Phytic acid	899.93±10.29	346.21±2.00	0.001	967.77±0.23	85.50±0.40	0.001	352.72±0.40	73.87±0.03	0.001
Oxalic acid	48.66±0.05	33.54±0.05	0.001	590.00±5.00	42.50±1.00	0.001	339.79±0.27	65.88±0.20	0.001

*Paired T test used. Significance set at p<0.05

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