Antioxidant and Type 2 Diabetes Related Functional Properties of Phytic Acid Extract from Kenyan Local Food Ingredients: Effects of Traditional Processing Methods

Catherine N. Kunyanga, Jasper K. Imungi, Michael W. Okoth, Hans K. Biesalski & Vellingiri Vadivel

Department of Food Science, Nutrition and Technology, University of Nairobi, Nairobi, Kenya
Institute for Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany

Available online: 06 Sep 2011

To cite this article: Catherine N. Kunyanga, Jasper K. Imungi, Michael W. Okoth, Hans K. Biesalski & Vellingiri Vadivel (2011): Antioxidant and Type 2 Diabetes Related Functional Properties of Phytic Acid Extract from Kenyan Local Food Ingredients: Effects of Traditional Processing Methods, Ecology of Food and Nutrition, 50:5, 452-471

To link to this article: http://dx.doi.org/10.1080/03670244.2011.604588

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.tandfonline.com/page/terms-and-conditions

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.
Antioxidant and Type 2 Diabetes Related Functional Properties of Phytic Acid Extract from Kenyan Local Food Ingredients: Effects of Traditional Processing Methods

CATHERINE N. KUNYANGA, JASPER K. IMUNGI, and MICHAEL W. OKOTH
Department of Food Science, Nutrition and Technology, University of Nairobi, Nairobi, Kenya

HANS K. BIESALSKI and VELLINGIRI VADIVEL
Institute for Biological Chemistry and Nutrition, University of Hohenheim, Stuttgart, Germany

Emerging scientific evidences reveal that phytic acid has several positive effects on human health. The antioxidant and type 2 diabetes related enzyme inhibition properties of phytic acid extract prepared from raw and traditionally processed local grains and vegetables collected from Kenya were evaluated. Phytic acid content of raw grains and vegetables ranged between 2.81–3.01 and 0.29–3.23 g/100 g DM, respectively. The phytic acid extract from raw samples revealed 59%–89% of DPPH radical scavenging capacity, 27–3,526 mmol Fe(II)/g extract of reducing power, 20%–72% of α-amylase inhibition activity and 8%–91% of α-glucosidase inhibition activity. Cooking and roasting improved the antioxidant and health relevant functionality of phytic acid extracts obtained from Kenyan local vegetables and grains, respectively.

KEYWORDS local Kenyan foods, phytic acid, antioxidant activity, type 2 diabetes relevant functionality, traditional processing methods
Phytic acid (myo-inositol 1,2,3,4,5,6-hexakis-dihydrogen phosphate; IP₆) is the major storage form of phosphorus in plants, comprising 60%–90% of total phosphorus. It is ubiquitous seed constituent, composing 1.0%–5.0% of edible portion of all nuts, cereals, legumes and oil seeds (Champ 2002). Recently, there has been an increasing interest on the health protective biochemical functions of phytic acid and its application in the prevention of oxidative stress related diseases in human beings (Khattab et al. 2010; Verghese et al. 2006). There is growing scientific and epidemiological evidence associating phytic acid intake with reduced incidences of cardiovascular disease, cancer and age-related degenerative processes (Jariwalla et al. 1990).

Phytic acid was found to play a major role in the treatment of cancer, hypercholesterolemia, hypercalcuria and kidney stones (Grases et al. 2000; Plaami 1997; Vucenik and Shamsuddin 2003). Subsequently, the beneficial properties of phytic acid such as antioxidant (Graf and Eaton 1990; Khattab et al. 2010), anticarcinogenic (Shamsuddin 1995; Verghese et al. 2006), anti-calcification (Grases et al. 2000; Schlemmer et al. 2009) and hypoglycemic or hypolipidemic (Jariwalla et al. 1990; Lee et al. 2006, 2007) were evaluated, which have great importance in human nutrition. Therefore, studies on the positive effects of dietary phytic acid have revived research about its significance in human nutrition and health.

Phytic acid is predominantly present in unprocessed foods, but can be degraded during thermal processing and resulting in a broad range of inositol phosphates, which are consumed in the diet (Khattab and Arntfield 2009). The profiles and quantities of phytic acid in foods are affected by processing due to phytic acid’s highly reactive nature, which may also affect its functional properties (Egounlety and Aworh 2003; Schlemmer et al. 2009). Hydrolysis of phytic acid during soaking, cooking and fermentation is reported to be mainly due to phytic acid-degrading activity of phytase enzyme that is naturally present in plants. Consequently, food processing is crucial in affecting the phytic acid content through dephosphorylation and regulation of composition of partially phosphorylated myo-inositol phosphate ester with health benefits.

The mean daily intake of phytic acid for infants (∼1½–2½ years) and school-age children (7–9 years) is 1,066 and 2,390 mg, respectively in Kenya (Schlemmer et al. 2009). Even though reports of studies on the nutritional value of local Kenyan foods are abounding (Neumann et al. 2003), the levels of phytic acid and its functional properties in these foods remain unexplored. Moreover, the effects of commonly applied domestic processing techniques on the concentration of phytic acid and its functionality have been studied even less. Therefore, the present study was aimed to evaluate the antioxidant potential and type 2 diabetes related enzyme inhibition characteristics of phytic acid extract obtained from raw and processed Kenyan local food samples with a view to identify the most elite food sample(s) as well as suitable processing method(s) in preserving the functionalities.
MATERIALS AND METHODS

Chemicals

The chemicals used include sodium phytate (Ref: 238-242-6); 2,2’-Diphenyl-1-picryl hydrazyl (DPPH, Ref. No.: 217-591-8); 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ, Ref. No. 3682-35-7), Butylated Hydroxytoluene (Ref. No. 204-881-4), α-amylase (Ref. No. 9001-19-8), and α-glucosidase (Ref. No. 9001-42-7) were obtained from Sigma Chemical, USA, and all other chemicals were purchased from Merck, Darmstadt, Germany.

Sample Collection and Preparation

The samples were collected on the basis of common food samples used by vulnerable groups in Nairobi, Kenya. Vulnerable groups are population groups whose immune system is compromised or depressed nutritionally, medically, or socially and require provision of extra, nutritionally high quality foods in addition to the general ration to rehabilitate or prevent deterioration in their conditions. They include: the malnourished; children under 5 years of age; Pregnant and lactating women; Medical referrals (malnourished adults in cases of vitamin or mineral deficiencies, TB, diabetes, cancer, AIDS); people living with HIV/AIDS (PLWHA’s) refugees; internally displaced persons (IDP’s) and socially vulnerable groups (orphans or unaccompanied children; the elderly, the disabled and any individuals separated from family and unable to fend for themselves).

Samples included cereals such as finger millet (Eleusine coracana L. Gaertn. P-224) and amaranth grain (Amaranthus cruentus L.); legumes such as pigeonpea (Cajanus cajan (L.) Millsp. Kat/Mbaazi 3) and field bean (Dolichos pupureum L. Kat/DL-3); oil seeds such as groundnut (Arachis hypogea L.), pumpkin seed (Cucurbita maxima Duchesne ex Lam.) and sunflower seed (Helianthus annuus L. PAN 7369). The vegetables selected were pumpkin (Cucurbita maxima L.), butternut (Juglans cinerea L.), sweet-potato (Ipomoea batatas [L.] Lamk. SPK 004), and leafy vegetables such as drumstick leaves (Moringa oleifera L.), amaranth leaves (Amaranthus hybridus L.) and pumpkin leaves (Cucurbita maxima Lam.). The cereals, legumes, oil seeds and vegetable samples (1 kg each) were randomly obtained from Kenya Agricultural Research Institute (KARI), Kenya as well as different parts of Kenya from the agricultural fields. Then they were mixed to obtain a representative sample, which was then subdivided into three portions for different treatments with three replicates.

Processing of Cereals, Legumes, and Oil Seeds

The grains (i.e., cereals, legumes, and oil seeds) were randomly divided into three batches for each. The first batch was stored as such without any
treatment and regarded as raw samples. The second batch of samples (100 g each) was washed with tap water and then soaked in distilled water (200 ml) for 8 h in the dark at 25 ± 1°C and cooked at 90°C–95°C for 120 min in fresh distilled water in the ratio of 1:4. The third batch of samples was roasted in an open-pan iron container to a golden brown color for 30 min using traditional charcoal burner at 150°C with continuous stirring to ensure even roasting. After roasting, the samples were cooled to room temperature.

Processing of Vegetables and Leafy Vegetables

The fresh vegetables were randomly divided into three batches and the first batch was stored as such without any treatment and regarded as raw samples. The second batch of samples (100 g of edible portion) were cut into small pieces or cubes (approximately 1cm in size) and washed under running tap water, then cooked in 200 ml of distilled water at 90°C–95°C for 5 min for all samples, except sweet potatoes, pumpkin and butternut which are cooked for 15 min. The third batch of vegetables were each cut as above and blanched by immersing in boiling water for 5 min.

All the raw, cooked and blanched samples were dried in hot-air oven at 50°C for 6 h (As per the traditional method practiced by the vulnerable group in Kenya for the preparation of food) and then milled using a laboratory mill (Hammer mill, type DFH48, No. 282521/UPM 6000, Switzerland) and sieved (.1mm) to obtain fine flour for further analyses.

Analysis of Phytic Acid

The phytic acid was extracted from raw and differentially processed samples by taking 1 g of defatted flour with 10 ml of 2.4% HCl and incubated for 10 min in ultra-sonic bath followed by magnetic stirrer for 30 min. Then the contents were centrifuged at 13,000 × g for 5 min and the supernatant was collected. Similarly, the residue was re-extracted twice and all the supernatants were pooled together and made up to a known volume with distilled water. The extract was purified by using an anionic-exchange column chromatography (.7 cm × 15 cm) containing .5 g of anion-exchange resin (100–200 mesh, chloride form; AG1-X4, Bio-Rad Co., California, USA) and phytic acid was eluted with 2 M HCl.

Phytic acid was quantified according to Latta and Eskin method (1980). The partially purified extract (500 μl) was diluted to 2.5 ml with distilled water and 1 ml of Wade reagent (.03% FeCl₃.6H₂O and .3% sulfosalicylic acid) was added. The contents were vortexed and centrifuged at 3,500 × g for 5 min and the absorbance of the supernatant was measured at 500 nm using a UV-Vis Spectrometer (Perkin-Elmer, Lambda 35, USA). The phytic acid content was calculated by using the standard curve prepared with synthetic sodium phytate and expressed as g/100 g on dry weight.
basis. The extract was frozen at –80°C for overnight and dried in lyophilier (Virtis Freeze mobile 25 EL, New York, USA) for 8 h and finally the residue was weighed and the total dry yield of extract was calculated. The extract was re-dissolved in distilled water the ratio of one milligram of extract per milliliter of solvent and used for further analysis.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of phytic acid extract was analyzed by following the method of Sanchez-Moreno, Larrauri, and Saura-Calixto (1998). The aqueous extract (.1 ml) of each sample was added to 3.9 ml (.025 g/L) of DPPH solution. BHT (1 mg/ml) was used as a positive control and pure DPPH solution alone was used as negative control. The solutions were incubated at room temperature (25°C) for 30 min and at the end of incubation period the decrease in absorbance was determined at 515 nm with a Spectrophotometer. The radical scavenging activity of the tested samples was measured as a decrease in the absorbance of DPPH radical and was calculated by using the equation,

\[
\text{DPPH radical scavenging activity (\%)} = (1 - \frac{A_{\text{samples}}}{A_{\text{negative control}}}) \times 100.
\]

Ferric Reducing Antioxidant Power (FRAP)

The reducing property of the aqueous extract was estimated according to the procedure described by Pulido, Bravo, and Saura-Calixto (2000). FRAP reagent (900 μl), prepared freshly and incubated at 37°C, was mixed with 90 μl of distilled water and 30 μl of test sample or distilled water (for the reagent blank) or BHT as positive control. The test samples and reagent blank were incubated at 37°C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mM TPTZ solution (2,4,6-Tris (2-pyridyl)-s-triazine) in 40 mM HCl plus 2.5 ml of 20 mM FeCl₃.6H₂O and 25 ml of .3 M acetate buffer, pH 3.6 (16.8 g of glacial acetic acid, MW 60.05 and 2.69 g of sodium acetate trihydrate, MW 136.1 dissolved in 1 L of distilled water). At the end of incubation the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Different concentrations of Fe(II) ranging from 100 to 2000 μM (FeSO₄.7H₂O) were used for the preparation of the calibration curve.

α-Amylase Inhibition Activity

The α-amylase inhibition activity was measured following the method of Worthington (1993). One-hundred microlitre of 1% starch solution in .02 M sodium phosphate buffer (pH 6.9) was added to 100 μl of aqueous extract of each sample in 100 μl of .02 M sodium phosphate buffer (pH 6.9) containing
α-amylase solution (1 unit liberates 1.9 μl of maltose from starch in 1 min at pH 6.9 and temperature 25°C), and was incubated at 25°C for 30 min. After the incubation, the reaction was stopped with 1.0 ml of dinitrosalicylic acid reagent. The test tubes were incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted to 10-fold with distilled water and the absorbance was measured at 540 nm. The readings were compared with the control, which contained buffer instead of sample extract. Based on the absorbance value, the percent inhibition activity was calculated for all the samples.

α-Glucosidase Inhibition Activity

The α-glucosidase inhibition activity was determined according to the method described by Worthington (1993). One-hundred microlitre of aqueous extract and 100 μl of .1 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1 unit/ml) were taken in tubes and incubated at 25°C for 5 min. After the pre-incubation, 100 μl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in .1 M phosphate buffer (pH 6.9) was added to each tube and the reaction mixture was incubated at 25°C for 5 min. After the incubation period the aliquots were diluted with 10-fold distilled water, and the absorbance readings recorded at 405 nm and compared to a control that had 100 μl of buffer solution in place of the extract. The results were calculated and expressed as percentage of α-glucosidase inhibition.

Statistical Analysis

All analyses were performed in triplicate (n = 3), and the data was presented as means standard error of deviation (± SEM). The results obtained were analyzed by using two-way ANOVA to determine the significant differences between the experimental batches by taking the raw samples as control. GraphPad PRISM® version IV software, San Diego, California, was used for statistical analysis.

RESULTS AND DISCUSSION

Phytic Acid Content

Phytic acid contents for the grain samples analyzed in the present study ranged from 2.57 to 3.01 g/100 g DM with the finger millet and pumpkin seeds exhibiting the highest contents (figure 1). The phytic acid content of the oil seeds (2.81–3.01 g/100 g) in the present study showed similar values to those reported for canola oil seed (2.16–3.75 g/100 g) in a previous study (Khattab et al. 2010). Additionally, earlier reports indicate that phytic acid occurs in various foods at different concentrations ranging...
from .1 to 6.0 g/100 g (Verghese et al. 2006). Data obtained from this study was within the range of phytic acid values reported for cereals such as maize, wheat, rice, millet, sorghum and barley (.06–3.35 g/100 g) as well as legumes such as kidney beans, pinto beans, black eye beans, broad beans, cowpeas and peas (.22–2.90 g/100 g) in a review on phytic acid in foods by Schlemmer and others (2009). The variation in range of phytic acid contents in different grains reflect the great number of botanical; varieties of seeds; various growing environmental or climatic conditions and different stages of seed maturation.

Among the vegetables investigated in this study, the phytic acid content was found to range from .29 to 3.23 g/100 g (figure 2). Sweetpotato and butternut were found to possess highest levels of phytic acid among the vegetables, while the leafy vegetables had significantly low phytic acid content. The phytic acid content of the leafy vegetables (.29–2.95 g/100 g) evaluated in this study was found to be similar to that reported for green leafy vegetable such as amaranth (Amaranthus tricolor), bathua (Chenopodium album), fenugreek (Trigonella foenumgrecum) and spinach (Spinacia oleracea) (129.67–234.50 mg/100 g) grown in India (Yadav and Sehgal 2003). The variation in phytic acid content of vegetables could be due to varietal differences and growing locations.

**DPPH Radical Scavenging Activity**

The antioxidant potential is among the most remarkable properties of phytic acid and is mainly based on complexing iron between three phosphate
groups through inhibition of hydroxyl radical formation from \( \text{H}_2\text{O}_2 \) via Fenton reaction by the \( \text{Fe}^{2+}\)-phytic acid complex (Schlemmer et al. 2009). The ability of phytic acid extracted from raw and processed grains to scavenge the stable synthetic DPPH free radical is shown in table 1. The phytic acid extract of raw grains exhibited promising levels of the DPPH free-radical scavenging activity (78%–85%), but lower than the synthetic antioxidant BHT (94%).

Among the studied grains, the phytic acid extract of finger millet and pumpkin seeds, which had high levels of phytic acid were observed to also exhibit significantly high radical scavenging activity. The phytic acid extracted from oil seeds in the current study exhibited excellent radical scavenging activity (83%–85%) which was in agreement with earlier values reported for canola oil seed (63%; Khattab et al. 2010). Phytic acid occurring in grains acts as an antioxidant through the formation of chelates with prooxidant transition metals. In the past, due to this property, phytic acid was considered as an antinutrient due to the mineral-binding activity. However, recently phytic acid has been reported to reduce the risk for colon and breast cancer in animals (Graf and Eaton 1990), a desirable positive health effect. This is because phytic acid, by complexing iron, may bring about a favorable reduction in the formation of hydroxyl radicals in the colon.

The ability of phytic acid extracted from different vegetables to donate hydrogen was estimated using the DPPH free radical. The radical-scavenging activity of phytic acid of the vegetables ranged from 59% to 89% with butternut squash (89%), pumpkin (87%) and sweet potato (87%) exhibiting the
<table>
<thead>
<tr>
<th>Food samples</th>
<th>DPPH assay (%)</th>
<th></th>
<th>FRAP Assay (mmol Fe[II]/g extract)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw seeds</td>
<td>Cooked seeds</td>
<td>Roasted seeds</td>
<td>Raw seeds</td>
</tr>
<tr>
<td>Finger millet</td>
<td>84.67 ± 2.85</td>
<td>74.33 ± 0.88</td>
<td>77.67 ± 2.33</td>
<td>52.52 ± 5.47</td>
</tr>
<tr>
<td>Amaranth grain</td>
<td>81.33 ± 0.33</td>
<td>72.33 ± 0.33</td>
<td>71.67 ± 1.20</td>
<td>26.86 ± 0.96</td>
</tr>
<tr>
<td>Pigeon pea</td>
<td>81.33 ± 0.33</td>
<td>82.67 ± 0.33</td>
<td>72.67 ± 2.40</td>
<td>45.11 ± 3.50</td>
</tr>
<tr>
<td>Field bean</td>
<td>78.00 ± 0.58</td>
<td>75.67 ± 3.18</td>
<td>83.00 ± 1.00</td>
<td>77.94 ± 10.06</td>
</tr>
<tr>
<td>Ground nuts</td>
<td>83.33 ± 0.33</td>
<td>83.33 ± 5.69</td>
<td>84.67 ± 2.85</td>
<td>44.50 ± 0.58</td>
</tr>
<tr>
<td>Pumpkin seeds</td>
<td>85.33 ± 0.88</td>
<td>65.00 ± 2.00</td>
<td>81.67 ± 1.76</td>
<td>109.31 ± 6.84</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>85.33 ± 1.67</td>
<td>66.33 ± 1.76</td>
<td>86.00 ± 3.51</td>
<td>106.59 ± 4.21</td>
</tr>
<tr>
<td>BHT</td>
<td>97 ± 2.31</td>
<td></td>
<td></td>
<td>2370 ± 173.37</td>
</tr>
</tbody>
</table>

**Note.** Values are mean ± SEM of three separate determinations (n = 3). Values in the same row with different alphabet superscripts are significantly different (p < .05).
highest activities (table 2). Similar levels of DPPH radical scavenging activity were reported for sweet potato (87%) grown in Japan (Oki et al. 2002). The radical-scavenging activity of pumpkin (87%) of the current study was similar to the value (78.4%) reported for the same sample cultivated in Malaysia (Azizah et al. 2009). The butternut squash, pumpkin, and sweet potato were also observed to possess higher phytic acid content among the presently studied vegetables and thus exhibiting high antioxidant property.

FRAP Activity

Phytic acid forms an iron chelate, which accelerates Fe^{2+}-mediated oxygen reduction yet blocks iron-driven hydroxyl radical generation and suppresses lipid peroxidation (Khattab et al. 2010). The FRAP activity of phytic acid extract of the raw grains ranged from 25.86 to 109.31 mmol Fe[II]/g extract with the highest reducing power observed in pumpkin seeds and sunflower seeds, which also exhibited high DPPH radical scavenging activity (table 1). Xu and Chang (2007) reported phytic acid levels similar to those observed for legumes of the current study (78–119 mmol Fe[III]/g) in other legumes such as yellow pea (54–159 mmol Fe[III]/g), green pea (62–116 mmol Fe[II]/g), black bean (113–1103 mmol Fe[III]/g), chick pea (73–113 mmol Fe[II]/g), soy bean (127–993 mmol Fe[III]/g) and red kidney beans (285–922 mmol Fe[III]/g) collected from USA.

The FRAP activity of phytic acid extract of the presently studied vegetables ranged from 42.59 to 3526.46 mmol Fe[II]/g with the highest reducing power recorded in the leafy vegetables, particularly in the amaranth and pumpkin leaves (table 2). Certain vegetables notably potato, kale, cabbage, cucumber, brussel sprouts and spinach have been reported to have slightly lower reducing power (ranging from 24 to 265 mmol Fe[II]/g; Halvorsen et al. 2002) than the presently studied vegetables.

α-Amylase Inhibition Activity

Foods that result in low blood glucose response have been shown to have immense nutritional significance in the prevention and/or management of type 2 diabetes mellitus. Inhibition of α-amylase by phytic acid lowers the blood glucose response and may prove to be useful in the clinical management of hyperglycemia and diabetes (Jariwalla et al. 1990; Lee et al. 2006). The in vitro reduction of starch digestion was positively correlated with the myo-inositol phosphate concentration and negatively with the number of phosphate groups on the myo-inositol ring (Lee et al.). The α-amylase inhibition activity of phytic acid of presently analyzed grains was ranged between 55% and 72% with the pumpkin seeds and finger millet exhibiting the highest level (table 3).
<table>
<thead>
<tr>
<th>Food samples</th>
<th>DPPH assay (%)</th>
<th>FRAP Assay (mmol Fe[II]/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw vegetables</td>
<td>Cooked vegetables</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>86.67 ± 4.33</td>
<td>84.00 ± 3.22</td>
</tr>
<tr>
<td>Butternut squash</td>
<td>88.67 ± 2.60</td>
<td>75.67 ± 3.71</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>87.33 ± 2.33</td>
<td>79.33 ± 4.09</td>
</tr>
<tr>
<td>Drumstick leaves</td>
<td>61.33 ± 5.33</td>
<td>59.67 ± 0.88</td>
</tr>
<tr>
<td>Pumpkin leaves</td>
<td>59.33 ± 1.20</td>
<td>65.67 ± 1.76</td>
</tr>
<tr>
<td>Amaranth leaves</td>
<td>81.67 ± 1.45</td>
<td>74.00 ± 1.16</td>
</tr>
<tr>
<td>BHT</td>
<td>97 ± 2.31</td>
<td></td>
</tr>
</tbody>
</table>

Note. Values are mean ± SEM of three separate determinations (n = 3). Values in the same row with different alphabet superscripts are significantly different (p < .05).
<table>
<thead>
<tr>
<th>Food samples</th>
<th>α-Amylase inhibition (%)</th>
<th>α-Glucosidase inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw grains</td>
<td>Cooked grains</td>
</tr>
<tr>
<td>Finger millet</td>
<td>65.67 ± 3.53</td>
<td>60.33 ± 3.84</td>
</tr>
<tr>
<td>Amaranth</td>
<td>65.00 ± 3.22</td>
<td>65.00 ± 3.06</td>
</tr>
<tr>
<td>Pigeon pea</td>
<td>55.33 ± 2.33</td>
<td>55.67 ± 2.60</td>
</tr>
<tr>
<td>Field beans</td>
<td>56.67 ± 5.24</td>
<td>48.00 ± 4.16</td>
</tr>
<tr>
<td>Groundnuts</td>
<td>57.00 ± 3.00</td>
<td>63.67 ± 2.19</td>
</tr>
<tr>
<td>Pumpkin seeds</td>
<td>71.67 ± 2.60</td>
<td>46.00 ± 2.52</td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>56.00 ± 2.52</td>
<td>53.67 ± 2.60</td>
</tr>
<tr>
<td>Acarbose</td>
<td>15.68 ± 2.41</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± SEM of three separate determinations (n = 3). Values in the same row with different alphabet superscripts are significantly different (p < .05).
The α-amylase inhibition activity of phytic acid extract of the presently studied vegetables ranged from 24% to 67% with sweet potato and drumstick leaves exhibiting the highest activity (table 4). Kaushik and colleagues (2010) reported varying degree of hypoglycemic and anti-hyperglycemic activity of commonly consumed vegetables in India. The authors revealed that sweet potatoes exhibited high antidiabetic activity and are recommended for the management of type 2 diabetes mellitus, in addition to other food samples. All the presently analyzed phytic acid extracts inhibited α-amylase action, indicating that phytic acid would have a potential function to suppress the elevation of postprandial glucose level from starch. It should be noted that to date there are no studies to reveal the inhibition of α-amylase enzyme activity reducing the starch digestion by phytic acid from Kenyan local food samples, so to our knowledge this is the first report.

α-Glucosidase Inhibition Activity

The phytic acid interaction with starch and divalent metals has been shown to result in low glycemic index and also reduces the participation of iron in oxidation metal mediated effects related with low diabetes (Schlemmer et al. 2009). Phytic acid extract of the presently investigated food ingredients caused significant inhibition of α-glucosidase enzyme activity (8%—91%; tables 3 and 4). The extent of inhibition of α-glucosidase of food samples was directly related to their phytic acid content which could be synergistic to their potential therapeutic effect on post-meal blood glucose level. For example, finger millet and butternut squash, which had the highest phytic acid content in the presently investigated food ingredients, were the most effective inhibitors of α-glucosidase. Generally, the grains were observed to exhibit greater α-glucosidase inhibition activity when compared to the vegetables. The α-glucosidase inhibition activity of phytic acid extract of the grains ranged from 14% to 91% with pigeon pea and finger millet demonstrating the highest activity (table 3). Epidemiological studies have reported the low incidence of diabetes in populations consuming millets in their regular diets (Shobana, Sreerama, and Malleshi 2009), which might be due to their potential α-glucosidase inhibition activity as revealed by the present study.

α-Glucosidase inhibition activity of the presently investigated vegetables falls between 8% and 66% with the butternut and amaranth leaves showing the highest level (table 4). Recent studies have reported that certain local foods such as pumpkin, corn, beans and sweet potato exhibit α-glucosidase inhibition activity and have the potential to reduce hyperglycemia-induced pathogenesis (Kwon et al. 2007). To our knowledge, this is the first study reporting the α-glucosidase inhibition activity of these local vegetables.
<table>
<thead>
<tr>
<th>Food samples</th>
<th>α-Amylase inhibition (%)</th>
<th>α-Glucosidase inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumpkin</td>
<td>62.33 ± 2.02</td>
<td>11.67 ± 3.28</td>
</tr>
<tr>
<td>Butternut squash</td>
<td>20.33 ± 4.33</td>
<td>66.00 ± 1.53</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>68.00 ± 1.53</td>
<td>8.00 ± 0.01</td>
</tr>
<tr>
<td>Drumstick leaves</td>
<td>67.33 ± 2.19</td>
<td>36.33 ± 0.67</td>
</tr>
<tr>
<td>Pumpkin leaves</td>
<td>62.00 ± 0.58</td>
<td>40.67 ± 0.67</td>
</tr>
<tr>
<td>Amaranth leaves</td>
<td>64.00 ± 2.08</td>
<td>48.00 ± 2.08</td>
</tr>
<tr>
<td>Acarbose</td>
<td>15.68 ± 2.41</td>
<td>44.54 ± 3.89</td>
</tr>
</tbody>
</table>

Note: Values are mean ± SEM of three separate determinations (n = 3). Values in the same row with different alphabet superscripts are significantly different (p < .05).
Effects of Processing

SOAKING AND COOKING OF GRAINS

Phytic acid is quite stable up to 100°C and it cannot be easily denatured by heat treatment such as that applied in household cooking, roasting, pressure cooking and fermentation. However, enzymatic degradation of phytic acid by phytase occurs naturally during food processing and may result in strong phytic acid hydrolysis (Schlemmer et al. 2009). Phytases are naturally present in plant parts, which involved in enzymatic dephosphorylation of phytic acid. In the present study, soaking and cooking caused no significant losses in the phytic acid content, FRAP activity, DPPH radical scavenging activity and α-amylase inhibition effect of some of the grains (figure 1, tables 1 and 3). However, significant losses were observed in the phytic acid content of sunflower seeds (16%); DPPH radical scavenging activity of pumpkin and sunflower seeds; FRAP values and α-amylase inhibition activity of pumpkin seeds; and α-glucosidase inhibition activity of finger millet, pigeonpea and field bean. Similarly, the phytic acid content of cowpea, pea and kidney bean were significantly reduced during soaking (43%–49%) and cooking (31%–69%; Khattab and Arntfield 2009).

Soaking of cereals and legumes can promote diffusion of phytic acid into soaking water, which was attributed to the fact that phytic acid in dried grains exist wholly as a water soluble salt such as sodium/potassium phytate. A significant phytic acid reduction can be realized by discarding the soaked water since phytic acid is water soluble, in addition to the action of endogenous phytases. Xu and Chang (2008) have also reported a decrease in DPPH activity of boiled green-pea, yellow-pea and chickpea due to leaching of soluble antioxidant components into boiling water.

In addition, soaking and cooking of grains resulted in significant increases in the phytic acid content of field bean, FRAP activity of the amaranth grain, pigeonpea and groundnuts, as well as α-glucosidase inhibition activity of amaranth grain. Similarly, increased phytic acid content after soaking of soybean, ground bean, and cowpea for 12–14 h has been observed by Egounlety and Aworh (2003). During food processing, phytic acids can be dephosphorylated to produce degradation products such as myo-inositol pentakis (IP₅), tetrakis (IP₄), tris- (IP₃), bis- (IP₂) and monophosphate (IP₁).

ROASTING OF GRAINS

Roasting did not cause significant reduction in the phytic acid content in the grain samples, except the pumpkin (15%) and sunflower seeds (13%; figure 1). Similarly, Khattab and Arntfield (2009) reported significant reduction (35%–40%) of phytic acid content during roasting of cowpea, pea, and kidney bean which were attributed partly due to the formation of insoluble complexes between phytic acid and other components.
Roasting also did not cause any significant losses in the DPPH radical-scavenging activity, FRAP values and \( \alpha \)-amylase inhibition activity of all the grains investigated in the present study (tables 1 and 3). However, significant losses were noticed in the \( \alpha \)-glucosidase inhibition activity of finger millet, field bean and sunflower seeds. Significant increases were observed in the phytic acid content of amaranth grain; FRAP activity of finger millet, amaranth grain, pigeonpea, and groundnuts; \( \alpha \)-amylase inhibition activity of field bean and sunflower seeds; as well as \( \alpha \)-glucosidase inhibition activity of pigeonpea, groundnuts and pumpkin seeds during roasting. Reports from other studies indicate that roasting caused no significant losses in the phytic acid content of small red kidney beans as well as \( \alpha \)-amylase inhibition activity of both red peanuts and kidney beans, but losses were observed after roasting of red peanuts (Ejigui et al. 2005).

COOKING OF VEGETABLES

Cooking degrades phytic acid to myo-inositols with a lower number of phosphate groups with similar beneficial effects like those of IP6, as it has been found that myo-inositols are involved in cell signaling in mammalian cells (Vucenik and Shamsuddin 2003). Cooking caused no significant reduction in the phytic acid content of most of the vegetables, except losses observed in the drumstick leaves (42%) and pumpkin leaves (71%; figure 2). Similarly, it has been reported that cooking of some green leafy vegetables such as amaranth (Amaranthus tricolor), bathua (Chenopodium album), fenugreek (Trigonella foenum grecum), and spinach (Spinacia oleracia) did not cause significant changes their phytic acid content (Yadav and Sehgal 2003). Cooking did not cause any significant losses in the DPPH radical-scavenging activity, FRAP activity, \( \alpha \)-amylase and \( \alpha \)-glucosidase inhibition activities of all the vegetables (tables 2 and 4). During cooking endogenous phytases are inactivated by heat and made ineffective to breakdown the phytic acid, which can then only be degraded by high temperature (Yadav and Sehgal 2003). However, slight losses were noticed in \( \alpha \)-amylase inhibition activity of drumstick leaves as well as \( \alpha \)-glucosidase inhibition activity of butternut while significant increases were observed in FRAP activity of all the leafy vegetables and \( \alpha \)-amylase inhibition activity of butternut. Phytic acid is relatively heat stable and hence significant heat destruction of phytic acid during cooking should not expected to occur. Therefore, considerable phytic acid dephosphorylation during cooking only takes place either by discarding the cooking water or by enzymatic phytic acid hydrolysis due to the action of the intrinsic plant phytases during the early part of the cooking phase.
BLANCHING OF VEGETABLES

Blanching caused no significant loss of phytic acid content in most of the vegetables investigated in this study, except reduction was noticed in the drumstick leaves (figure 2). However, significant losses were observed in the DPPH radical-scavenging activity of pumpkin, butternut squash, and sweet potato; FRAP activity of pumpkin and amaranth leaves; \( \alpha \)-amylase inhibition activity of pumpkin and drumstick leaves; as well as \( \alpha \)-glucosidase inhibition activity of butternut squash, pumpkin, and amaranth leaves (tables 2 and 4). Blanching of some selected vegetables such as bathua, fenugreek and spinach leaves from India was observed to reduce the phytic acid level due to the rupture of cell walls that causes soluble phytic acid to leach out into the blanching medium (Yadav and Sehgal 2003). Significant increases were observed in the phytic acid content and FRAP activity of amaranth leaves and pumpkin, as well as \( \alpha \)-amylase inhibition activity of butternut squash and \( \alpha \)-glucosidase inhibition activity of sweet potatoes.

Recent studies have shown conflicting findings with relation to effects of blanching on the antioxidant activity of vegetables. Reports by several authors have demonstrated that blanching significantly influence the antioxidant activity of vegetables and the effects were not consistent in different foods with some showing increases in antioxidant activity while others noticing decreases (Amin, Norazaidah, and Hainida 2006; Turkmen, Sari, and Velioglu 2005; Xu and Chang 2008). The increased antioxidant potential caused by processing could be due to the improvement of naturally occurring compounds or the formation of novel compounds possessing high antioxidant activity. However, leaching of antioxidant compounds into water during boiling and degradation or formation of complexes can also contribute to low antioxidant activity.

CONCLUSION

To exploit the health-promoting functionalities of the locally available, culturally acceptable, and economically viable foods, emphasis should be focused on the bioactive compounds present in plant foods consumed by vulnerable groups in Kenya. In this context, the present study revealed that certain selected Kenyan local grains and vegetables contain high levels of phytic acid, associated with high antioxidant and type 2 diabetes related enzyme inhibition properties. A good relationship has been observed between phytic acid content and antioxidant as well as type 2 diabetes related properties. Finger millet and amaranth leaves exhibited excellent type 2 diabetes related enzyme inhibition properties while pumpkin and sunflower seeds showed promising antioxidant activity. Cooking of vegetables and roasting of grains improved the functionality of phytic acid
extract. The presently studied local grains and vegetables could contribute to the significant supply of dietary antioxidants to prevent oxidative-stress related chronic diseases including type 2 diabetes, which is a growing problem among vulnerable groups in Kenya. The elite food sources and potential processing methods identified from the present investigation could be considered further for the formulation of supplementary foods with health protective effects for the vulnerable groups of Kenya. Formulation of a low-cost, fortified, supplementary diet from locally available food ingredients with health beneficial bioactive compounds, using appropriate small to medium–scale production technologies in community settings, can help to meet the nutritional needs of vulnerable groups. This strategy may also decrease significantly the prevalence of malnutrition as well as incidence of chronic diseases like type 2 diabetes. Therefore, by taking account of nutritive profiles and health beneficial role of bioactive compounds like phytic acid, formulation of supplementary foods with therapeutic value using the presently studied local food ingredients for vulnerable groups in Kenya is in progress.

ACKNOWLEDGMENT

The authors wish to thank the German Academic Exchange Services (Deutscher Akademischer Austausch Dienst - DAAD) for financial support, which was part of the funding for PhD study of the first author.

REFERENCES


