Evaluation of Indigenous Grains from the Peruvian Andean Region for Antidiabetes and Antihypertension Potential Using In Vitro Methods

Lena Galvez Ranilla, Emmanouil Apostolidis, Maria Ines Genovese, Franco Maria Lajolo, and Kalidas Shetty

ABSTRACT The health-relevant functionality of 10 thermally processed Peruvian Andean grains (five cereals, three pseudocereals, and two legumes) was evaluated for potential type 2 diabetes-relevant antihyperglycemia and antihypertension activity using in vitro enzyme assays. Inhibition of enzymes relevant for managing early stages of type 2 diabetes such as hyperglycemia-relevant α-glucosidase and α-amylase and hypertension-relevant angiotensin I-converting enzyme (ACE) were assayed along with the total phenolic content, phenolic profiles, and antioxidant activity based on the 1,1-diphenyl-2-picrylhydrazyl radical assay. Purple corn (Zea mays L.) (cereal) exhibited high free radical scavenging-linked antioxidant activity (77%) and had the highest total phenolic content (8 ± 1 mg of gallic acid equivalents/g of sample weight) and α-glucosidase inhibitory activity (51% at 5 mg of sample weight). The major phenolic compound in this cereal was protocatechuic acid (287 ± 15 µg/g of sample weight). Pseudocereals such as Quinoa (Chenopodium quinoa Willd) and Kañiwa (Chenopodium pallidicaule Aellen) were rich in quercetin derivatives (1,131 ± 56 and 943 ± 35 µg [expressed as quercetin aglycone]/g of sample weight, respectively) and had the highest antioxidant activity (86% and 75%, respectively). Andean legumes (Lupinus mutabilis cultivars SLP-1 and H-6) inhibited significantly the hypertension-relevant ACE (52% at 5 mg of sample weight). No α-amylase inhibitory activity was found in any of the evaluated Andean grains. This in vitro study indicates the potential of combination of Andean whole grain cereals, pseudocereals, and legumes to develop effective dietary strategies for managing type 2 diabetes and associated hypertension and provides the rationale for animal and clinical studies.

KEY WORDS: • α-amylase inhibitory activity • Andean grains • angiotensin I-converting enzyme • antioxidant activity • α-glucosidase inhibitory activity • hypertension • phenolic phytochemicals • type 2 diabetes

INTRODUCTION

The Andean region forms a long chain of mountains over 7,000 km long that stretch from Southern Venezuela to the South of Chile. The longest north–south mountain range in the world, the Andes encompass a tremendous range of ecosystems and are home to a rich variety of plant and animal species and human communities.1 For example, in Peru, there are 25,000 species of plants, which corresponds to 7–10% of the total species existing in the world. Of this high number, 38 species are domesticated, including tubers, roots, grains, fruits, and vegetables, whereas a large number of medicinal and ornamental plants still have not been domesticated.2 Andean grains have been cultivated for thousands of years since pre-Colombian times and include the “pseudocereals” Quinoa (Chenopodium quinoa Willd.), Kañiwa (Chenopodium pallidicaule Aellen), and Amaranth (Amaranthus caudatus L.), the Andean lupin or “Tarwi” (Lupinus mutabilis Sweet), and several pigmented varieties of corns (Zea mays L.). The purple corn is the most representative and consumed among these cereals. Nutritionally, Andean pseudocereals are often closer to the ideal protein balance than any other common cereal grains, being at least equal to milk in protein quality. In particular, they have high lysine content. Therefore, in order to obtain a suitable amino acid profile to cover the needs of the human diet, it is not necessary to combine them with other crops such as legumes, rich in lysine but lacking in methionine and cysteine, as happens for other cereals.3 Further, the Andean legume “Tarwi” (L. mutabilis Sweet) has the highest protein and lipid content of all the domesticated species of lupin, and its protein value is comparable to that present in soybeans.4


Address correspondence to: Kalidas Shetty, Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, MA 01003, USA. E-mail: kalidas@foodsci.umass.edu
Andean crops have shown a high level of resistance to drought, frost, salinity, pests, and diseases. Therefore, Andean grains may be promising with regard to their diversity of phenolic secondary metabolites (phytochemicals) synthesized to counteract such adverse climatic and growing conditions. Phytochemicals and in particular phenolic metabolites are bioactive non-nutrient plant compounds found in fruits, vegetables, grains, and other plant foods. These metabolites not only are involved in various stress and defense responses in plants (primary function) but also are currently being linked to the reduction of the risk of major chronic diseases when consumed via diet. For example, plant polyphenols, a large group of natural antioxidants, are important candidates linked to the protective effects of vegetables and fruits against cancer and cardiovascular diseases.

Among Andean grains, flavonoids such as kampferol and quercetin glycosides were found in the “Kancolla” variety of C. quinoa seeds. Similarly, Rastrelli et al. identified new flavonol triglycosides in seeds of C. pallidicaule. Further, the Andean purple corn had shown interesting health-related properties such as antioxidant, antimicrobial, and prevention of obesity and amelioration of hyperglycemia in mice, which were mainly related to its rich content of anthocyanins. Further, other phenolic compounds such as phenolic acids and flavonols, also identified in purple corn, showed potent antimutagenic properties in vitro. These have potential for managing chronic disease emergence resulting from globalization and narrower choices of the food supply that are high in refined and soluble carbohydrate sources.

The effects of globalization and urbanization on the food supply have influenced dietary patterns and lifestyle behaviors among traditional population groups throughout the world. In a recent study carried out in Peru, the migration of Peruvian Amerindian women from the rural Cuzco region (Andes) to urban areas in Lima (coast), resulting in different degrees of physical activity (high in Cuzco, low in Lima) and altitudes of living (highland in Cuzco, lowland in Lima), was associated with the increased risk for obesity and cardiovascular diseases. In Latin America, as elsewhere, traditional food patterns rich in complex carbohydrates, micronutrients, fiber, and phytochemicals are being replaced with diets high in animal products and refined carbohydrates and oils, situations that are leading to the rapid increase of obesity and chronic diseases such as type 2 diabetes, hypertension, and cardiovascular diseases.

Type 2 diabetes is a condition associated with elevated glycemic index, insulin resistance, and eventually declining pancreatic function that results in absolute or relative insulin deficiency. The importance of poor glycemic index management leading to insulin resistance as part of the natural history of diabetes is its association with metabolic syndrome: the human condition characterized by the presence of coexisting traditional risk factors for cardiovascular disease, such as hypertension, dyslipidemia, glucose intolerance, and obesity, in addition to nontraditional cardiovascular disease risk factors, such as inflammatory processes and abnormalities of the blood coagulation system. Managing hyperglycemia and hypertension through a whole grain diet could be effective on early-stage management of type 2 diabetes.

Andean grains provide a potential resource for South America and other regions of the developing world because their exceptional nutritional characteristics with the potential for alleviating malnutrition. Specific research on phenolic bioactive compound-linked health benefits from these grains may also show their potential for managing hyperglycemia and hypertension linked to type 2 diabetes and related cardiovascular complications.

Based on the above rationale, the objective of this study was to investigate the health-relevant functionality of phenolic phytochemicals related to type 2 diabetes-relevant antihyperglycemia and antihypertension potential of Andean grains using in vitro enzyme models. Therefore, phenolic profiles, antioxidant activity, and in vitro inhibition of enzymes relevant for managing early stages of type 2 diabetes such as hyperglycemia-relevant α-glucosidase and α-amylase and hypertension-relevant angiotensin I-converting enzyme (ACE) were investigated.

**MATERIALS AND METHODS**

**Materials**

Dry grains including pseudocereals and cereals (corn) were obtained from an Andean local market in Arequipa, Peru. Seeds of L. mutabilis Sweet H-6 and SLP-1 were provided by the Legume and Cereal Program of Agraria University (Lima, Peru) (Table 1).

<table>
<thead>
<tr>
<th>Code</th>
<th>Common name</th>
<th>Scientific name</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kañiwa</td>
<td>C. pallidicaule Aellen</td>
<td>Pseudocereal</td>
</tr>
<tr>
<td>2</td>
<td>Red Quinoa</td>
<td>C. quinoa Willd</td>
<td>Pseudocereal</td>
</tr>
<tr>
<td>3</td>
<td>Kiwicha</td>
<td>A. caudatus L.</td>
<td>Pseudocereal</td>
</tr>
<tr>
<td>4</td>
<td>Corn 1 (purple pigmented)</td>
<td>Z. mays L.</td>
<td>Cereal</td>
</tr>
<tr>
<td>5</td>
<td>Corn 2 (yellow, purple-red mottled)</td>
<td>Z. mays L.</td>
<td>Cereal</td>
</tr>
<tr>
<td>6</td>
<td>Corn 3 (yellow, red blotched)</td>
<td>Z. mays L.</td>
<td>Cereal</td>
</tr>
<tr>
<td>7</td>
<td>Corn 4 (half yellow-half red)</td>
<td>Z. mays L.</td>
<td>Cereal</td>
</tr>
<tr>
<td>8</td>
<td>Corn 5 (purple mottled)</td>
<td>Z. mays L.</td>
<td>Cereal</td>
</tr>
<tr>
<td>9</td>
<td>Tarwi SLP-1</td>
<td>L. mutabilis Sweet</td>
<td>Legume</td>
</tr>
<tr>
<td>10</td>
<td>Tarwi H-6</td>
<td>L. mutabilis Sweet</td>
<td>Legume</td>
</tr>
</tbody>
</table>
Porcine pancreatic α-amylase (EC 3.2.1.1), baker’s yeast α-glucosidase (EC 3.2.1.20), and rabbit lung ACE (EC 3.4.15.1) were purchased from Sigma Chemical Co. (St. Louis, MO). Unless noted, all chemicals also were purchased from Sigma.

**Extract preparation**

Dry grains (10 g) were added to 15 mL of distilled water and soaked overnight at 25 °C in order to facilitate the subsequent thermal processing. Pseudocereals and legumes were thermally processed by autoclaving at 120 °C for 10 minutes. A longer autoclaving time was required (20 minutes) in the case of corn samples. After the thermal treatment, 85 mL of distilled water was added to the cooked grains, and the mixture was homogenized for 1 minute using a Waring® laboratory blender (Waring Laboratory, Torrington, CT) set on “high” speed. The homogenate was centrifuged at 9,300 × g for 10 minutes before each in vitro assay. Extractions were performed in duplicate.

**Total phenolics assay**

The total phenolics were determined by the Folin-Ciocalteu method as modified by Shetty et al. In brief, 1 mL of the grain extract was transferred into a test tube and mixed with 1 mL of 95% ethanol and 5 mL of distilled water. To each sample 0.5 mL of 50% (vol/vol) Folin-Ciocalteu reagent was added and mixed. After 5 minutes, 1 mL of 5% Na₂CO₃ was added to the reaction mixture and allowed to stand for 60 minutes. The absorbance was read at 725 nm. The standard curve was established using various concentrations of gallic acid in 95% ethanol, and results were expressed as mg of gallic acid equivalents/g of sample weight.

**Antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition assay**

The DPPH scavenging activity was determined by an assay modified by Kwon et al. A volume of 50 μL of grain extract diluted with 50 μL of 0.1 M potassium phosphate buffer (pH 6.9) and 100 μL of 0.1 M potassium phosphate buffer (pH 6.9) containing α-glucopyranoside solution in 0.1 M potassium phosphate buffer (pH 6.9) was incubated in 96-well plates at 25 °C for 10 minutes. After preincubation, 50 μL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 5 minutes. Before and after incubation, absorbance readings were recorded at 405 nm by a microplate reader (Thermomax; Molecular Devices Co., Sunnyvale, CA) and compared to a control that had 50 μL of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as percentage of inhibition and was calculated as follows:

\[
Inhibition = \frac{A_{540\,\text{control}} - A_{540\,\text{extract}}}{A_{540\,\text{control}}} \times 100
\]

**α-Amylase inhibition assay**

The α-amylase inhibitory activity was determined by an assay modified from the Worthington Enzyme Manual. A total of 500 μL of each grain extract and 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25 °C for 10 minutes. After preincubation, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25 °C for 10 minutes. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted after adding 15 mL of distilled water, and absorbance was measured at 540 nm. Sample blanks (buffer instead of enzyme solution) and a control (buffer in place of sample extract) were included as well. The α-glucosidase inhibitory activity was calculated according to the equation below:

\[
Inhibition = \frac{A_{405\,\text{control}} - A_{405\,\text{extract}}}{A_{405\,\text{control}}} \times 100
\]

**ACE inhibition assay**

ACE inhibition was performed by a method modified by Kwon et al. A volume of 50 μL of grain extract was incubated with 200 μL of 0.1 M NaCl-borate buffer (0.3 M NaCl, pH 8.3) containing 2 μU of ACE solution at 25 °C for 10 minutes. After preincubation, 100 μL of a 5.0 mM substrate (hippuryl-histidyl-leucine) solution was added to the reaction mixture. Test solutions were incubated at 37 °C for 1 hour. The reaction was stopped with 150 μL of 0.5 N HCl. The hippuric acid formed was detected and quantified by high-performance liquid chromatography (HPLC). A volume of 5 μL of sample was injected using an Agilent ALS 1100 autosampler into an Agilent 1100 series HPLC apparatus (Agilent Technologies, Palo Alto, CA) equipped with a DAD 1100 diode array detector. The solvents used for the gradient were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% for 5 minutes and then decreased to 0% for the next 5 minutes (total run time,
18 minutes). The analytical column used was Agilent Zorbax SB-C18 (250×4.6 mm inner diameter) with packing material of 5 μm particle size at a flow rate of 1 mL/minute at room temperature. During each run the absorbance was recorded at 228 nm, and the chromatogram was integrated using the Agilent Chemstation enhanced integrator for detection of liberated hippuric acid. Pure hippuric acid was used to calibrate the standard curve and retention time. The percentage of inhibition was calculated considering the area of the hippuric acid peak according to the equation below:

\[
\text{Inhibition} = \frac{\text{Area}_{\text{control}} - (\text{Area}_{\text{sample}} - \text{Area}_{\text{sample blank}})}{\text{Area}_{\text{control}} - \text{Area}_{\text{blank}}} \times 100
\]

**HPLC analysis of phenolic profiles**

The grain extracts (2 mL) were filtered (pore size, 0.2 μm). A volume of 5 μL of sample was injected using an Agilent ALS 1100 autosampler into an Agilent 1100 series HPLC apparatus equipped with a DAD 1100 diode array detector. The solvents used for gradient elution were (A) 10 mM phosphoric acid (pH 2.5) and (B) 100% methanol. The methanol concentration was increased to 60% for the first 8 minutes and to 100% over the next 7 minutes, then decreased to 0% for the next 3 minutes, and was maintained for the next 7 minutes (total run time, 25 minutes). The analytical column used was Agilent Zorbax SB-C18 (250×4.6 mm inner diameter) with packing material of 5 μm particle size at a flow rate of 1 mL/minute at room temperature. During each run the absorbance was recorded at 306, 333, and 525 nm, and the chromatogram was integrated using the Agilent Chemstation enhanced integrator. Pure standards of quercetin aglycone and protocatechuic and p-coumaric acids in 100% methanol were used to calibrate the standard curves and retention times.

**Statistical analysis**

Two extractions were performed for each sample, and all in vitro analysis were carried out six times (n = 12). In the case of HPLC analysis, the experiments were performed at least in triplicates. Results were expressed as mean ± standard deviation values. Data were subjected to one-way analysis of variance, with means compared using Tukey’s test (P < 0.05), and Pearson correlations were calculated according to the Statistica software package version 5.0 (StatSoft, Tulsa, OK).

**RESULTS**

**Total phenolics, antioxidant activity, and HPLC phenolic profiles**

Figure 1 shows the total phenolic contents from Andean pseudocereals, cereals, and legumes correlated to their DPPH radical scavenging-linked antioxidant activity.

The total phenolic content in water extracts of cooked Andean grains varied from 0.80 ± 0.07 to 8 ± 1 mg/g. Sample 4 (purple corn) exhibited the highest total phenolic content (8 ± 1 mg of gallic acid/g), followed by the two legumes (samples 9 and 10) (L. mutabilis Sweet) (4 ± 0.2 and 4.1 ± 0.3 mg/g for SLP-1 and H-6 cultivars, respectively). Both Kaniwa and Quinoa (samples 1 and 2, respectively), which belong to the same botanical genus (Chenopodium), showed similar total phenolic contents (2.3 ± 0.1 and 2.8 ± 0.1 mg/g, respectively). On the other hand, the other partially pigmented corn seeds (samples 5–8) had lower phenolic levels (from 0.9 ± 0.1 to 1.1 ± 0.1 mg/g).

The antioxidant activity based on the DPPH radical inhibition assay ranged from 6% to 86% and had a moderate but statistically significant correlation with the total phenolic contents (r = 0.53, P < .05) (Table 2). Although Quinoa (sample 2) did not have the highest total phenolic levels, it exhibited the highest antioxidant activity among all Andean grains (86%). Kaniwa and purple corn were the second best grains with high antioxidant activity (75% and 77%, respectively).

Except for purple corn, the antioxidant activity among corn samples was moderate (from 24% to 36%). Lupin cultivars (sample 9 and 10) showed similar trends in their total phenolic contents. However, the ability to inhibit the

**Table 2. Pearson Correlation Coefficients for Antioxidant Activity (AA), α-Glucosidase Inhibitory Activity (GLUC), and ACE Inhibition of Andean Pseudocereals, Cereals, and Legumes**

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>GLUC</th>
<th>ACE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>0.53*</td>
<td>0.60*</td>
<td>0.20</td>
</tr>
<tr>
<td>AA</td>
<td>0.50*</td>
<td>-0.13</td>
<td>-0.09</td>
</tr>
<tr>
<td>GLUC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TP, total phenolics.

*P < .05.

*For a 5-mg sample weight (n = 120).

*For a 5-mg sample weight (n = 102).
against weight. Andean purple corn exhibited the highest inhibition activities ranged from 11% to 51% at 5 mg of sample dose-dependent manner (Fig. 2). The inhibitory activities a 3 and 4 (samples 6 and 7, respectively).

<table>
<thead>
<tr>
<th>Code</th>
<th>Common name</th>
<th>Quercetin derivatives*</th>
<th>Protocatechuic acid</th>
<th>p-Coumaric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kiwicha</td>
<td>943 ± 35</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Quinoa</td>
<td>1,131 ± 56</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>Corn 1</td>
<td>ND</td>
<td>287 ± 15</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>Corn 3</td>
<td>ND</td>
<td>ND</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>7</td>
<td>Corn 4</td>
<td>ND</td>
<td>ND</td>
<td>34 ± 5</td>
</tr>
</tbody>
</table>

Data are mean ± SD values. ND, not detected.

*Expressed as quercetin aglycone.

DPPH free radical was higher in the H-6 cultivar (42%) than in the SLP-1 lupin cultivar (24%). The pseudocereal known as “Kiwicha” (A. caudatus L.) had the lowest antioxidant activity (8%), which was proportional to its low total phenolic content.

Individual phenolic compounds detected by HPLC-diode array detector in water extracts of autoclaved Andean grains are shown in Table 3. Chenopodium species (samples 1 and 2) were characteristic for their content of quercetin derivatives (943–1,132 µg/g, expressed as quercetin aglycone). Only protocatechuic acid was detected in purple corn, whereas low levels of p-coumaric acid were found in corns 3 and 4 (samples 6 and 7, respectively).

**α-Glucosidase, α-amylase, and ACE-I inhibitory activities**

All Andean grains inhibited the yeast α-glucosidase in a dose-dependent manner (Fig. 2). The α-glucosidase inhibitory activities ranged from 11% to 51% at 5 mg of sample weight. Andean purple corn exhibited the highest inhibition against α-glucosidase (51%), followed by corn 3 (sample 6) (40%) and Quinoa (sample 2) (30%). Kiwicha (A. caudatus L.), the pseudocereal with the lowest total phenolic contents and antioxidant activity, also had the lowest α-glucosidase inhibitory activity (11%). Legumes showed moderate α-glucosidase inhibitory activities. However, the H-6 lupin cultivar (sample 10) was more effective against the α-glucosidase enzyme than the SLP-1 lupin cultivar (sample 9) (26% and 19%, respectively). According to Table 2, statistical correlations between total phenolic contents and α-glucosidase inhibitory activities and between the antioxidant activity and α-glucosidase inhibitory activities were significant (r = 0.60 and r = 0.50, respectively, P < .05). However, comparing both Pearson correlation coefficients, the ability of Andean grain water extracts for inhibiting α-glucosidase in vitro was better correlated to their total phenolic contents than to their free radical scavenging-linked antioxidant activity.

The α-amylase inhibitory activity was also screened among Andean grains. However, no α-amylase inhibitory activity was detected in any of the grains.

Only legumes (lupin cultivars) exhibited a significant inhibitory effect on ACE at a dose of 5 mg of sample weight (Fig. 3), but no ACE inhibition was detected when lower doses of sample were assayed. Likewise, no significant differences (P > .05) between the ACE inhibitory activities of both lupin cultivars were observed (52% for SLP-1 and H-6 cultivars). According to Pearson correlations (Table 2), neither the total phenolic contents nor the antioxidant activities were correlated with the ACE inhibitory activities of lupin cultivars.

**DISCUSSION**

**Total phenolic content, antioxidant activity, and HPLC phenolic profiles**

Overall, the total phenolic contents in Andean grains were proportional to their antioxidant activity, and this fact...
appeared to be correlated to the presence of specific phenolic compounds detected by HPLC-diode array detector.

Among analyzed Andean grains, purple corn (Z. mays L.) was the most relevant grain because of its highest total phenolic contents and DPPH radical scavenging-linked antioxidant activity, followed by both legumes (*L. mutabilis* Sweet) and the pseudocereals Kãiâwa (*C. pallidicaule* Aellen) and Quinoa (*C. quinoa* Willd.).

Purple corn, rich in anthocyanins, has been cultivated for centuries in the Andean region. In Peru, people consume a typical drink made by boiling purple corn called “Chicha Morada,” which is believed by folklore use to improve health. The average anthocyanin content of whole purple corn from Peru was 1,640 mg/100 g of fresh weight, higher than fresh blueberries (73–430 mg/100 g of fresh weight). Anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-glucoside, and peonidin-3-glucoside have been identified in a purple corn extract from Peru. However, other non-anthocyanin phenolic compounds such as quercetin derivatives and ferulic and p-coumaric acid derivatives were also detected. The free radical scavenging activity of purple corn has been associated to its high content of anthocyanins. In the current study, only protocatechuic acid was identified, and no anthocyanins were detected when the autoclaved purple corn water extract was analyzed by HPLC-diode array detector at 525 nm.

It is well known that anthocyanins are sensitive to heat and can easily convert to the colorless chalcone form during heating. Sadilova *et al.* indicated that successive deglycosylation reactions represent the initial steps of degradation of anthocyanins following thermal treatment at pH 1, yielding the corresponding aglycones. The latter would then be cleaved into a phenolic acid and a phenolic aldehyde. Further, Jing and Giusti observed a consistent decrease of protein at 100°C in a purple corn water extract indicating a possible protein denaturation at high temperatures, which could result in anthocyanin complexation and precipitation leading to a declining total anthocyanin content.

Cywandin 3-glucoside was identified as the major anthocyanin present in purple corn together with its acylated equivalents. Under neutral conditions and high temperatures, cyanidin glycosides are consecutively hydrolyzed mainly to protocatechuic acid. However, other minor compounds such as 2,4-dihydroxybenzoic acid and 2,4,6-trihydroxybenzoic acid are also formed. Similarly, Sadilova *et al.* showed that thermal treatment lead to the formation of protocatechuic acid and phloroglucinaldehyde as a result of cyanidin-3-glucoside deglycosylation and further cleavage of the respective aglycone. This rationale would explain the detection of protocatechuic acid as the main individual phenolic compound in water extracts of thermally processed purple corn analyzed in this study.

Currently, a purple corn extract from powders obtained by extraction of ground purple corn in a 60% aqueous ethanol solution, filtering, and further spray-drying is being commercially sold for the nutraceutical market. This powder has been shown to contain high levels of anthocyanins with potential health benefits attributed to those compounds. However, these powder preparations failed to consider that purple corn is still traditionally consumed as a beverage prepared by boiling purple corn in water. According to results in this study, the high total phenolic levels and DPPH radical scavenging-linked antioxidant activity of water extracts from thermally processed purple corn seeds could be correlated with the presence of protocatechuic acid formed as result of anthocyanin degradation by the autoclaving process.

*L. mutabilis* Sweet, also known as “pearl lupin” or “Tarwi,” is a valuable legume protein that has been found in the Andean region since ancient times. With regard to its phenolic contents and antioxidant activities assayed in this study, both cultivars (SLP-1 and H-6) showed high total phenolic contents linked to moderate antioxidant activities, although no specific phenolics using current methods were detected by HPLC-diode array detector in water extracts from thermally processed lupin seeds. No reports on the antioxidant activity of *L. mutabilis* have been published to date. However, results obtained for other lupin species indicated that the antioxidant activity (measured by the photochemiluminescence assay) had no correlation with the total phenolic contents in seeds of *Lupinus angustifolius*. On the other hand, Tsaliki *et al.* observed a high correlation between total phenolic contents and phospholipid levels with the antioxidant activity of *Lupinus albus* ssp. *graecus*, using the method based on the coupled oxidation of β-carotene and linoleic acid. In this study, lupin antioxidant activity based on the DPPH radical inhibition was moderately proportional to their total phenolic contents, suggesting the contribution of other non-phenolic compounds, probably carotenoids, most likely degraded and solubilized after thermal treatment. Carotenoids such as zeaxanthin and β-carotene have been found in *L. mutabilis* and *Lupinus luteus* in higher concentrations than in *L. angustifolius* and *L. albus*. Further, the ability of carotenoids to scavenge the DPPH free radical has been previously reported.

Quinoa (*C. quinoa* Willd.) is one of the oldest crops of the Andes, being cultivated for at least 7,000 years. Quinoa produces high-protein grains under ecologically extreme conditions, and this makes it important for the diversification of future agricultural systems, such as in high-altitude areas of the Himalayas and North Indian Plains. Kãiâwa (*C. pallidicaule* Aellen) provides exceptional nutritional qualities with high iron content in leaves and seeds. Also, its flour, like Quinoa, can be used by people with celiac disease, who cannot eat the gluten present in wheat, rye, barley, and oat. Kãiâwa cultivation is actually limited to the harvest and coldest areas of the Andes, above 3,800 m above sea level. Dini *et al.* reported the presence of kaempferol, quercetin glycosides, and a glycoside of vanillic acid in raw whole flour of the Quinoa seed variety Kancolla. Similarly, 10 flavonol glycosides, mainly quercetin, isorhamnetin, and kaempferol glycosides, have been identified in the raw whole flour from the seeds of *C. pallidicaule*. In the current study, only *Chenopodium* species exhibited high concentrations...
of quercetin derivatives, and this likely was correlated with their high radical scavenging-linked antioxidant activities. The strong free radical-scavenging properties of quercetin have been attributed to its special structural arrangement that includes an o-3',4'-dihydroxy moiety in the B ring and the presence of a 2,3-double bond in combination with both the 4-keto group and the 3-hydroxyl group in the C ring, for electron delocalization. Since these grains are commonly consumed after a cooking process, results from this study indicate for the first time the presence of quercetin derivatives in cooked grains of Quinoa and Kañiwa and their potential free radical scavenging-linked antioxidant activity.

Less colored corn seeds have been shown to contain free and esterified phenolic acids. Del Pozo-Insfran et al. identified six derivatives of ferulic acid (88.8–816 μg/g) along with the free form (2480 μg/g), p-coumaric acid (6.6 μg/g), two protocatechuic acid derivatives (4.2 and 14.2 μg/g), and gallic acid (3.9 μg/g) in a white corn genotype seed after its processing into nixtamal (cooked kernels). In addition, low concentrations of vanillic acid, -hydroxybenzoic acid, and protocatechuic acid have been found in yellow corn (3.7, 1.3, and 3.0 μg/g, respectively). In this study, only p-coumaric acid was identified in two partially pigmented corn seeds (yellow-red blotched and half yellow-half red, 22 and 34 μg/g of sample weight, respectively) after thermal processing by autoclaving.

α-Glucosidase and α-amylase inhibitory activity

Adult-onset type 2 diabetes is a metabolic disorder characterized by hyperglycemia leading subsequently to defects in insulin secretion, insulin action, or combinations. The long-term manifestation of type 2 diabetes can result in the development of microvascular or macrovascular complications.40 Early intervention in type 2 diabetes by using carbohydrate utilization inhibitors can delay the digestion of complex carbohydrates and intestinal uptake of glucose, thus significantly reducing postprandial glycemic and insulimimic excursions.41 Potential compounds are competitive inhibitors of pancreatic α-amylase for delaying ingested carbohydrate breakdown and of intestinal brush border α-glucosidases with the subsequent inhibition of uptake of glucose and oligosaccharides, resulting in lower glycemic index.42 However, because of their strong α-amylase inhibition, synthetic α-glucosidase inhibitors have undesirable side effects, such as flatulence, diarrhea, and abdominal cramping. In addition, some of them may increase the incidence of renal tumors and serious hepatic injury and acute hepatitis.43–46 Therefore, consumption of natural dietary inhibitors from constituents in food could be effective therapy at reduced cost for managing postprandial hyperglycemia with minimal adverse reactions as compared to modern synthetic pharmaceuticals.40

Results from the current study indicate that cooked Andean grains, especially purple corn, the yellow-red blotched corn (sample 6), and Quinoa (C. quinoa Willd), have potential for in vitro α-glucosidase inhibition with no effect on pancreatic α-amylase. Such profile has interesting functionality for potentially controlling intestinal glucose absorption and likely not generating side effects linked to high pancreatic α-amylase inhibitory activity.47

The significant correlation observed between the α-glucosidase inhibitory activity of Andean grains and their total phenolic contents (r = 0.60, P < .05) likely indicates the potential of Andean grain-linked phenolic compounds for inhibiting the hyperglycemia-relevant α-glucosidase enzyme. Thus, in purple corn and Quinoa, the α-glucosidase inhibitory activity may probably be associated with the contents of protocatechuic acid and quercetin derivatives, respectively. Previous reports with clonal herbal extracts reported high α-glucosidase inhibitory activity in vitro with specific standard phenolics. Pure protocatechuic acid on a constant weight and constant pH basis showed 56% inhibition, followed by quercetin (37%). Further, these standard phenolics did not have any inhibitory activity against porcine pancreatic α-amylase.48

The potential of purple corn for the amelioration of hyperglycemia and prevention of obesity and cholesterol has been correlated mainly with the presence of cyanidin-3-glycosides.12,48 Similarly, cholesterol reduction and increase of antioxidant capacity by continuous consumption of the anthocyanin-rich Andean purple corn have been recently demonstrated in hypercholesterolemic rats.49 Insights from the present study suggest that protocatechuic acid, the major degradation product of cyanidin-glycosides from purple corn following thermal treatment, could have potential in type 2 diabetes prevention as part of a dietary strategy. Recently, protocatechuic acid has been shown to exhibit other therapeutic effects such as induction of cell death in HepG2 hepatocellular carcinoma and a neuroprotective effect on rotenone-induced apoptosis of PC12 (a neuroendocrine cell line) via ameliorating the mitochondrial dysfunction.50,51

Kiwicha (A. caudatus L.), a highly nutritious Andean grain crop,52 was not interested with regard to its potential to inhibit key enzymes relevant for type 2 diabetes management. Further, cooked grains of Kiwicha showed low total phenolic contents and the lowest antioxidant activity. A previous study reported the α-amylase inhibitory activity in vitro of two varieties of Kiwicha (Oscar blanco and Victor red; 51% and 28%, respectively, at 25 μg/mL).53 However, analyses in this previous investigation were based on methanolic extracts of ground raw seeds.53 This would explain differences in results obtained in this study, where a thermal processing was applied prior to analysis of water extracts from Kiwicha grains to reflect consumption practices.

ACE-I inhibitory activity

Hypertension is a risk factor of cardiovascular disease and is also associated with long-term diabetes. ACE, a key enzyme involved in maintaining vascular tension, converts angiotensin I to angiotensin II, a potent vasoconstrictor and stimulator of aldosterone secretion by the adrenal gland.
Inhibition of ACE is considered a useful therapeutic approach in the treatment of high blood pressure in patients both with and without diabetes. Control of hypertension via modulation of ACE by dietary bioactive factors could be an important strategy to manage this risk factor. Among Andean grains, only lupin cultivars inhibited significantly the ACE enzyme, indicating their potential antihypertension activity, and therefore these legumes can be targeted for potentially combating this macrovascular complication of hyperglycemia.

Lupin species are of fundamental nutritional importance because their higher contents of protein comparable to that of soybean. L. mutabilis seeds have 48% dry weight of protein and 27% fat content, which is similar to soybeans but higher than other legumes. In general, legumes that contain a higher quantity of proteins produce higher levels of peptides by peptic digestion or by food processing, and such legumes show higher ACE inhibitory activities than cereals. Lupin protein isolates (L. albus) and its hydrolysates were found to inhibit in vitro the ACE to a degree of 0.7–43.4% (150 μg/mL of sample). However, the same authors reported later that the ACE inhibitory activities of lupin protein isolates (L. albus) decreased to 0–2.72% (150 μg/mL of sample) after high temperature treatments at 125°C. Results from this study indicate that cooked Andean lupin seeds (L. mutabilis) exhibit in vitro ACE inhibitory activity that was likely not associated with phenolics but more likely with its protein content. The role of protein in ACE inhibition has to be confirmed with further studies.

CONCLUSIONS

Type 2 diabetes has reached epidemic proportions, with the greatest increases in the developing countries of Africa, Asia, and South America. In Peru, the prevalence of type 2 diabetes varies from 1% to 8%, and the regions with the highest prevalence are Piura and Lima. Metabolic syndrome associated with type 2 diabetes is highly prevalent among Peruvian Andean Hispanics, particularly in older women. Further, type 2 diabetes was found in 17% of Peruvian women, with the highest incidence in the poorest areas. In Amerindian populations, one explanation may be the rapid transition from hunter-gatherer societies to an agricultural lifestyle, the transition to urbanization, and changes of traditional unrefined healthy diets towards refined high calorie diets devoid of protective phytochemicals. Paradoxically, Peru is also one of the countries with a wide diversity of plants and Andean crops potentially rich in health-relevant bioactive phenolic compounds. Research focused on functional benefits of local Andean crops for managing the above noted diet-linked chronic diseases and return to diversity of traditional whole food dietary patterns is urgently needed.

The current study using in vitro assays provides insights about the antihyperglycemia and antihypertension potential of thermally processed Andean pseudocereals, cereals, and legumes from Peru. Purple corn (Z. mays L.) (cereal) had the highest total phenolic content, free radical scavenging linked-antioxidant activity, and α-glucosidase inhibitory activity, characteristics likely correlated with its content of procatechuic acid. Pseudocereals such as Quinoa (C. quinoa Willd) and Kañiwa (C. pallidicaule Aellen), rich in quercetin derivatives, exhibited high antioxidant activities and moderate α-glucosidase inhibitory activities, whereas only legumes (L. mutabilis SLP-1 and H-6) had high ACE inhibition, indicating their potential antihypertension activity. Cooked Andean grains did not have α-amylase inhibitory activity. Therefore, the potential for managing glucose level, hypertension, and cellular redox status for prevention of type 2 diabetes and its complications without side effects linked to high α-amylase inhibition may be achieved by combining Andean cereals, pseudocereals, and legumes as a part of the traditional overall diet and food diversity. Such combinations of diet designs need to be further validated by animal and clinical studies.

AUTHOR DISCLOSURE STATEMENT

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