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RESEARCH ARTICLE

Changes in antinutrients, phenolics, antioxidant activities and in vitro α-glucosidase inhibitory activity in pumpkin leaves (*Cucurbita moschata*) during different domestic cooking methods

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Abstract Pumpkin leaves (*Cucurbita moschata*) were subjected to different household cooking methods (boiling, microwaving, steaming, and stir-frying) to evaluate their effect on antinutrients, phenolic compounds, antioxidant properties (ABTS, and DPPH) and in vitro α-glucosidase activity. All cooking methods studied significantly reduced the antinutrients and antioxidant activities, whilst phenolic compounds *p*-coumaric and ferulic acids significantly increased. The cooking methods reduced the oxalates by more than 50%, tannins by 47% and phytates by 79.22%. Steaming and boiling resulted in highest concentrations of *p*-coumaric (195.40 mg kg^{-1}) and ferulic acids (103.90 mg kg^{-1}) compared to other methods. Overall, boiled leaves retained the highest total phenolic compounds, whilst steamed leaves retained the highest antioxidant capacity. Raw pumpkin leaf extracts showed higher in vitro α-glucosidase inhibitory effects than the cooked leaves. Thus, cooking affected the inhibitory effect of in vitro α-glucosidase activity.

Keywords Oxalates · Indigenous leafy vegetables · Oxalates · α-glucosidase activity · Antioxidant capacity

Introduction

Pumpkin leaves (*Cucurbita moschata*) historically are eaten in Africa, where they are cooked together with young leaves, immature fruits, and flowers (Vorster et al., 2005). Ko et al. (2016) reported that pumpkin leaves are also popular in Korean cuisine as "*ssam,*" to wrap a piece of meat, such as pork or other filling accompanied by condiments, due to its characteristic taste and texture. A 100 g portion of fresh pumpkin leaves are rich in mineral sources and contain 38 mg of Mg, 39 mg of Ca, 15.90 mg of Fe and 3.15 g of protein, and low in cholesterol (Odhav et al., 2007). Whilst a 100 g portion of commonly consumed lettuce contains 27 mg of Ca, 10 mg of Mg, 0.64 mg of Fe and 1.13 g of protein (USDA food data base, 2020). However, the Fe content in 100 g dark leafy green vegetables such as spinach, collard greens and kale contain 1.50 mg, 1.06 mg and 1.59 mg respectively, which is lower compared to the pumpkin leaves. Therefore, daily consumption of 100 g portion of pumpkin leaves contributes to the Recommended Dietary Allowance (RDA) for Fe for 14–18 years old females (van Jaarsveld et al., 2014). Therefore, pumpkin leaves have the potential to be included in dietary diversification for nutritional benefits

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and to contribute to household food security (Jansen van Rensberg et al., 2007).

Pumpkin leaves are a healthy and functional vegetable, as they are rich in phenolics, flavonoids and β-carotene (Ko et al., 2016). Cha (2009) reported that the water extract of pumpkin leaves showed 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of 44.6% at the concentration of 20 mg mL^{-1}. Findings of Kim et al. (2011) stated that the ethanol extract of pumpkin leaves contained the highest total phenolic content (29.62 mg gallic acid equivalents g^{-1} dry weight), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)-radical scavenging activity, and ferric reducing antioxidant power (FRAP) compared to the other parts of the vegetable, such as skin, flesh, or seeds. Furthermore, pumpkin leaves contain cinnamic acid, *p*-hydroxybenzoic acid, gentisic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid and caffeic acid (Ko et al. 2016). Based on the findings of Ko et al. (2016), caffeic acid, *p*-coumaric acid, ferulic acid, and gentisic acid were regarded as the major phenolic compounds (Ko et al., 2016).

The staple food in developing countries include starch-based foods that are low in micronutrients, thus encouraging the consumption of traditional leafy vegetables, such as pumpkin leaves, can improve the daily micronutrient requirements. Apart from the aforementioned, pumpkins are easily produced with minimum fertilisers, have a short life cycle and are affordable for the consumers (Jansen van Rensberg et al., 2007). Production of pumpkin will help to *alleviate poverty* through employment creation and *income generation* in *rural* areas (Jansen van Rensberg et al., 2007).

Different household cooking techniques adopted by the rural communities enhance the taste and palatability of pumpkin leaves for consumption. Traditional household cooking techniques include boiling and steaming (Akdaş and Bakkalbaşı, 2017), but currently, microwave cooking and stir-frying are used for their convenience. Traditionally, *boiling* was the main *method of cooking* pumpkin leaves. Different cooking methods can affect the total polyphenols negatively due to the changes in different phenolic compounds (Managa et al 2020). At the same time, cooking can improve the extractability of phytochemicals due to the softening effect of the matrix (Palermo et al., 2014). Therefore, it is important to understand changes in the phytochemicals during different household cooking techniques to select the most suitable technique that can retain the antioxidant properties of pumpkin leaves. The concentration of antioxidant and radical scavenging activity after cooking depends on a variety of factors, including the nature of vegetables, form and period of boiling, boiling temperature, high temperature localization and stability (Jimenez-Monreal et al., 2009). Many research reports are available on the impact of household cooking on phenolics, antioxidant and radical-scavenging activity of exotic green leafy vegetables. However, these previous reports are limited for thermally cooked traditional vegetables. A previous study showed that steaming reduced the color difference *(ΔE)* in the pumpkin leaves compared to stir-frying, boiling, or microwaving (Mashiane et al., 2021). Sensory evaluation indicated the testing panel preferred the stir-fried leaves. Therefore, in light of the abovementioned, the objective of this study was to evaluate the changes in antinutrients, phenolic compounds, antioxidant activities and α-glucosidase inhibitory activity of pumpkin leaves during different domestic cooking methods.

Materials and methods

Chemicals

The following chemicals, HCl, vanillin, methanol, acetic acid, gallic acid, $C_2H_3NaO_2$, 2,4,6-tris (2-pyridyl)-1,3,5-triazine, Trolox, $FeCl_3$, 2,2′-azobis (2- amidinopropane) hydrochloride, 2,2′- azinobis (3-ethylbenzothiazoline-6-sulfonate), Na_2HPO_4-$7H_2O$, $NaH_2PO_4H_2O$, NaCl, 2,2′-azobis(2- amidinopropane) hydrochloride, 2,2-diphenyl-1-picrylhydrazyl. (DPPH), rat intestinal acetone powder, bovine serum albumin, *p*-nitrophenyl α-D-glucopyranoside, Na_2CO_3, protocatechuic acid, vanillic acid, syringic acid, ellagic acid, *p*-coumaric acid, ferulic acid, monohydrate ferric chloride sulfosalicylic acid, NH_4OH, H_2SO_4, $KMnO_4$ were purchased from Sigma Aldrich, Johannesburg, South Africa.

Sample preparation

The Tshiombo Irrigation Scheme in Vhembe District, Limpopo Province provided the pumpkin leaves. The dimension of the leaf was approximately 5 × 14.5 × 21 cm. The leaves were washed in running tap water soon after harvest, then transported to the laboratory, within 6 h, in cooler boxes at 10 °C. Leaves were stored at 5 °C in the cold room for 24 h prior to processing. The leaves were cut into pieces approximately 2.0 cm in diameter, which mimicked the traditional preparations, and mixed well for homogeneity. Each cooking technique included 20 replicates, each of which weighed 100 g.

Cooking methods

Traditionally boiling was the most preferred method of cooking pumpkin leaves (Jansen van Rensburg et al., 2004). Currently, traditional leaves are mixed and fried in

cooking oil (stir-frying) or steam blanched (with no addition of salt or other ingredients) (Jansen van Rensburg et al., 2004). Due to modern lifestyles, traditional dishes have undergone modification, therefore microwaving was included. The conclusion about the duration of each cooking method was based on interviews and literature-based evidence (Murador et al., 2016).

Boiling: pumpkin leaves (100 g) were boiled in 150 mL of water at 98 °C in a covered stainless-steel pot, on a moderate flame for 10 min, representing the traditional cooking method, drained and rapidly cooled on ice.

Stir-frying: 15 mL of virgin olive oil was placed in a preheated pan, 150 g of pumpkin leaves were added to the preheated pan and then stir-fried for 2 min at 120 °C. The samples were cooled quickly on ice.

Steaming: 100 g of pumpkin leaves were placed in stainless steamer pot compromising 250 mL of boiling water (98 °C) and steamed for 15 min, and then cooled on ice.

Microwave cooking: 100 g of pumpkin leaves were placed in a glass dish with 12 mL of water, and cooked in a microwave oven [Defy Appliances (Pty) Ltd, Midrand, South Africa] (household), working at 2450 MHz–900 W, for 10 min. After draining the sample, it was cooled rapidly on ice.

Temperatures during different cooking methods were measured using a food thermometer probe (Mingle Development Co., Ltd, Tong Fu Yu Industrial Park, Shenzhen, China). Ten replicates (each weighing 100 g) from each cooking technique and 10 replicate samples of raw fresh leaves (control) were snap frozen in liquid nitrogen and stored at − 80 °C for the analysis of antinutritive compounds, phenolic compounds, antioxidant activity and in vitro α-glucosidase activity.

Antinutritive compounds

The extraction and quantification of tannins, phytates and oxalate contents were made according to the methods previously described by Managa et al. (2020).

Tannin content: Snap frozen leaf samples (0.2 g) were mixed with 10 mL 1% HCl. The reaction mixture comprised 100 µL aliquot of the sample extract and 50 µL vanillin-HCl in methanol [5 mL of 8% HCl in methanol (5 mL) and 1% vanillin in methanol]. Tannin content was expressed as mg 100 g^{-1}.

Phytates content: An aliquot of 100 mL of 2.4% HCl was added to the snap frozen leaf samples (0.5 mg) to extract the phytates. The quantification was performed using Wade reagent (0.03 g monohydrate ferric chloride and 0.3 g sulfosalicylic acid in 100 mL distilled water). Phytates content was expressed mg 100 g^{-1}.

Oxalate content: Snap frozen leaf samples (0.1 g) were homogenised with 30 mL 2 M HCl to extract the insoluble oxalates. Soluble oxalates were extracted with distilled water using leaf samples (0.1 g). The CaC_2O_4 was precipitated by adding 5% $CaCl_2$, thereafter the pellets were collected, washed three times with 0.35 M NH_4OH and dissolved in 0.5 M H_2SO_4. The resulting solution (combined soluble and insoluble oxalates) was titrated against 0.1 M of $KMnO_4$ at 60 °C until an extremely faint pale pink color persisted for 15 min. The expression of oxalate concentration was as mg 100 g^{-1}.

Extraction and quantification of phenolic compounds in pumpkin leaves

Phenolic compounds were extracted, according to the methods previously described by Managa et al. (2019), by homogenising [Ultra Turrax (T25 digital ULTRA-TURRAX®, Lab limited, Surry, UK)] snap-frozen pumpkin leaves (0.2 g) in 2 mL of acidified methanol 80% methanol (v/v) [19.5% distilled water and 0.05% HCl] for 2 min. The resulting mixture was centrifuged for 15 min at 9500 rpm at − 4 °C. The supernatant was filtered through Whatman® no 1 filter paper.

High performance liquid chromatography (HPLC–DAD) was executed using a Shimadzu Prominence-i-LC-2030C 3D, Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), as described earlier by Mpai et al. (2018). Stock solutions of targeted standards references were prepared in the HPLC mobile phase at a concentration range of 0.025–0.300 mg mL^{-1} of gallic acid, protocatechuic acid, protocatechuic acid, vanillic acid, syringic acid, ellagic acid, *p*-coumaric acid, ferulic acid. All the chromatography operations were at ambient temperature and in triplicate.

Total antioxidant capacities

The antioxidant capacity of snap frozen raw and cooked pumpkin leaves was determined using ABTS (Antioxidant capacity) method, previously described by Managa et al. (2019). Pumpkin leaves (0.2 g) were extracted using 2 mL of sodium acetate buffer (pH 3.6) by mixing the sample extract (40 µL) with the radical solution (1960 µL) to measure the antioxidant activity at 734 nm for a period of 6 min. A calibration curve was constructed with Trolox as the standard and the antioxidant capacity was expressed as mg of TEAC g^{-1}.

Antioxidant scavenging activity was also determined using the DPPH assay, as previously described by Mpai et al. (2018). Fresh leaf sample (0.2 g) was mixed in 2 mL of methanol/water (2:4) extraction solution, followed by a series of dilutions to obtain concentrations of

0.9–60 mg mL^{-1}. An aliquot of 23 µL was drawn from each dilution and mixed with 210 µL of DPPH (90 µM), and the absorbance measured at 515 nm (BMG LABTECH GmbH, SpectroStar Nano, Ortenberg, Germany). Gallic acid was the standard, and the concentration of extract at which 50% inhibition was observed (IC_{50}), and the values of IC_{50} were expressed in mg mL^{-1}.

In vitro α-glucosidase activity

The inhibition of α-glucosidase activity was determined using the modified published method by Dewi et al. (2007). One mg of α-glucosidase (rat intestinal acetone powder) was dissolved in 100 mL of phosphate buffer (pH 6.8) containing 200 mg of bovine serum albumin. The reaction mixture consisting 100 µL of sample at varying concentrations (50–6.25 µg mL^{-1}) was premixed with 490 µL phosphate buffer pH 6.8 and 250 µL of 5 mM *p*-nitrophenyl α-D-glucopyranoside. After pre-incubating at 37 °C for 5 min, 250 µL α-glucosidase (0.15-unit mL^{-1}) was added and incubated at 37 °C for 15 min. The addition of 2000 µL Na_2CO_3 200 mM terminated the reaction. α-Glucosidase activity was determined spectrophotometrically at 540 nm on spectrophotometer UV–Vis (Shimadzu 265, Jepang, Japan) by measuring the quantity of *p*-nitrophenol released from *p*-NPG. Acarbose was used as the positive control of α-glucosidase inhibitor. The concentrations of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value. All tests were in triplicate.

Statistical analysis

A completely randomised design was adopted with 10 replicates per cooking technique, and the experiments repeated twice. One-way analysis of variance (ANOVA) tested for significant differences between the different cooking treatments. Means were compared amongst the treatments by the least significant difference (LSD) test, with $p < 0.05$, using the statistical programme Genstat for Windows, 13th Edition (2010) (VSN International Hempstead, UK).

Results and discussion

Antinutritive compounds

The investigation into the influence of domestic cooking methods on antinutrients was to select the most appropriate method that reduces the antinutritive compounds significantly. Tannins chelate metals with zinc and iron, making them unavailable for absorption in the gut. They also reduce protein digestibility by forming complexes with the protein or inhibiting digestive enzymes, as a result inhibiting their digestion and absorption (Olawoye and Gbadamosi, 2017). Foods with high tannin content have an astringent taste, reducing their palatability. Therefore, cooking may increase the palatability of the pumpkin leaves. A 100 g portion of raw pumpkin leaves contained a significantly ($p < 0.05$) higher concentration (51.49 mg) of tannins when compared to the leaves cooked with different methods (Table 1). All the domestic cooking methods used reduced the tannin content between 12.35 and 42.72%. Boiling caused the greatest loss (46.24%), which may be due to leaching of tannins into boiling water (Managa et al., 2019). Steaming resulted in the lowest reduction (12.35%) due to no direct contact between the vegetables and the water (Pellegrini et al., 2009). Similarly, boiling reduced the tannin in different traditional vegetables, such as *Solanum nigrum*, *Amaranthus hybridus, Amaranthus dubius Asystasia gangetica, Bidens pilosa, Oxygonum sinuatum, Chenopodium album, Emex australis, Guillemminea densa, Momordica balsamina, Physalis viscosa* and *Galinsoga parviflora* (Essack et al., 2017).

Oxalates in the diet are mainly from plants and plant products, and they chelate with calcium, thus reducing the mineral bioavailability (Akindahunsi and Salawu 2005). Oxalates are found in soluble form in plants, (sodium and potassium oxalate), as well as insoluble form (calcium and magnesium oxalate) (Chai and Liebman, 2005). The soluble form is available for mineral chelating. All the domestic cooking methods investigated reduced the concentration of oxalates by more than 50% (Table 1). Conventional boiling caused 81.89% loss of oxalates compared to the other three cooking methods. Similar decrease in oxalates were observed in boiled spinach (*Spinacia oleracea*), silverbeet (*Beta vulgaris* v. cicla) and rhubarb (*Rheum rhaponticum*) (Savage et al., 2000). Therefore, consumption of boiled pumpkin leaves may account for a lower risk, because soluble oxalate levels are reduced markedly during boiling. Moreover, the oxalate content in pumpkin leaves was much lower than the amount reported in green and red Swiss chard (964–1167 mg 100 g^{-1} on fresh weight basis) and spinach (1145 mg 100 g^{-1} on fresh weight basis) (Noonan and Savage, 1999). The content of solubleoxalate, and the methods used for cooking vegetables are important when making dietary recommendations for individuals predisposed to kidney stones. Foods were classified into three groups based on oxalate:calcium ratio (Noonan and Savage, 1999). Vegetables with oxalate:calcium ratio above 2 have high oxalate levels and are likely to bind with Ca from other foods consumed at the same time. Spinach leaves showed oxalate:calcium ratio greater than 2, indicating higher risk of binding with Ca, whilst green and red amaranth leaves showed the oxalate:calcium

Table 1 Effect of domestic cooking methods on antinutritive compounds in pumpkin leaves (*Cucurbita moschata*)

Household cooking methods	Antinutritive compounds (mg 100 g^{-1})					% Loss
	Tannins	% Loss	Oxalates	% Loss	Phytates	
Raw	51.49 ± 0.21*[a]		92.43 ± 0.17[a]		87.22 ± 0.17[a]	
Boiling	27.68 ± 0.31[e]	46.24 ± ± 0.31[a]	16.78 ± 0.17[e]	81.89 ± 0.44[a]	18.12 ± 0.27[e]	79.22 ± 0.17[a]
Steaming	45.13 ± 0.62[b]	12.35 ± 0.11[d]	44.86 ± 0.17[b]	51.46 ± 0.38[d]	68.98 ± 0.31[b]	20.91 ± 0.13[d]
Microwaving	29.45 ± 0.50[d]	42.72 ± 0.51[b]	22.01 ± 0.17[d]	76.18 ± 0.52[b]	22.97 ± 0.24[d]	73.66 ± 0.14[b]
Stir-frying	39.41 ± 0.41[c]	23.46 ± 0.54[c]	35.00 ± 0.17[c]	62.13 ± 0.57[c]	30.66 ± 0.47[c]	64.84 ± 0.68[c]

Averages were calculated based on ten replicate samples

Different letters in the same column refer to statistical difference [$p < 0.05$, least significant difference (LSD) test]

*Standard deviation

ratio less than 2, illustrating less risk of binding with Ca from other foods. In the future, total oxalate/total calcium (mEq) needs estimating for pumpkin leaves for dietary recommendations.

Cooking significantly reduced the phytate content. Steaming and boiling treatments resulted in the lowest (20.91%) and highest (79.22%) reduction of phytate content. Dietary phytates, in large, are also able to chelate minerals (Zn and Fe) and affect their bioavailability. At the same time, phytates also affect the protein and starch digestion (Agbaire, 2011). The presence of 4–9 mg of phytic acid in 100 g portion of leafy vegetable decreases the Fe absorption by 4 to fivefold (Akwaowo et al., 2000). Boiling different traditionally consumed leafy vegetables, such as *Solanum nigrum*, *Amaranthus hybridus, Amaranthus dubiu, and Momordica balsamina, for 10 min totally removed the phytates* (Essack et al., 2017). During boiling, the observed reduction in phytates was possibly due to the leaching of phytic acid into the boiling water caused by rupture of the cell wall (Yadav and Sehgal, 2002).

Identification and quantification of phenolic compounds

Raw pumpkin leaves contain gallic acid, protocatechuic acid, vanillic acid, syringic acid, ellagic acid, *p*-coumaric acid and ferulic acid (Table 2). Highest concentrations of protocatechuic acid (81.00 mg kg^{-1}) and ellagic acid (86.70 mg kg^{-1}) whilst trace amounts of *p*-coumaric acid and ferulic acid were detected in raw leaves. Overall, cooking increased the syringic, ellagic, coumaric and ferulic acid concentrations in pumpkin leaves compared to the raw leaves. All four cooking methods used in this study significantly ($p < 0.05$) reduced the concentrations of gallic acid and protocatechuic acid when compared to raw pumpkin leaves. Similarly, in white cauliflower, boiling, microwaving and stir-frying resulted in reduction in reduced gallic acid and protocatechuic acid (Ahmed and Ali 2013). Vanillic acid (42.20 mg kg^{-1}) was detected only in raw pumpkin leaves, and not found after boiling, microwaving, steaming or stir-frying. Boiling significantly led to the increased levels of syringic acid (37.90 mg kg^{-1}), ellagic acid (92.00 mg kg^{-1}) and ferulic acid (103.90 mg kg^{-1}) compared to the other domestic cooking methods, whilst microwaving caused a loss of ellagic acid content (49.90 mg kg^{-1}) in pumpkin leaves; furthermore, steaming caused a greater increase in the concentration of *p*-coumaric acid (195.40 mg kg^{-1}). Hydroxybenzoic acids (coumaric acid, ferulic acid, sinapic acid) are present in fruit and vegetables as a conjugated form. Hydroxybenzoic acid derivatives (gallic, syringic, protocatechuic and vanillic acids) are present in bound forms in complex structures with lignins and hydrolyzable tannins, carbohydrate, protein or as conjugated esters or amides. The observed increase in ellagic acid could be due to hydrolysis of ellagitannins (Muthukumaran et al., 2017). The higher temperature, duration of processing and the pH of the food matrix could have favoured the hydrolysis of ellagitannins (Muthukumaran et al., 2017). Therefore, the observed increase in the majority of the phenolic acids, except vanillic acid, during thermal processing is probably due to the release of bound phenolic compounds to free phenolic compounds from the leaves. Vanillic acid was thermally unstable and could have undergone decarboxylation to form other molecules (Ifie and Marshall, 2018). Results showed that same cooking methods had different effects on different phenolic compounds. Overall, microwaving showed lower concentrations of different phenolic compounds in pumpkin leaves. Compared to our observation, conventional boiling showed significant loss in different phenolic compounds in turnip greens (Francisco et al., 2010), mainly due to the release of phenolic compounds into the water due to the rupture of cellular tonoplast membrane and the cell wall due to boiling (Francisco et al., 2010). In our study, syringic acid, ellagic acid and ferulic acid clearly survived boiling, as shown in

Table 2 Impact of different household cooking methods on different phenolic compounds in pumpkin leaves (*Cucurbita moschata*)

Phenolic acids (mg kg^{-1})		RT (min)	*Mw*	Raw leaves	Boiling	Microwaving	Steaming	Stir-frying
Gallic acid	$C_7H_6O_5$	7.60	170.120	14.20 ± 0.5*[a]	11.40 ± 2.2[b]	6.40 ± 0.1[e]	9.30 ± 0.1[c]	8.90 ± 0.1[d]
Protocatechuic acid	$C_7H_6O_4$	11.60	154.120	81.00 ± 0.1[a]	53.30 ± 0.1[b]	27.10 ± 0.8[d]	51.30 ± 0.9[c]	23.30 ± 0.1[e]
Vanillic acid	$C_8H_8O_4$	14.50	168.140	42.20 ± 0.1	nd	nd	nd	nd
Syringic acid	$C_9H_{10}O_5$	15.50	198.170	14.50 ± 0.1[e]	37.90 ± 0.3[a]	15.20 ± 0.7[d]	33.300 ± 0.1[b]	17.30 ± 0.9[c]
Ellagic acid	$C_{14}H_6O_8$	16.30	302.197	86.70 ± 0.7[b]	92.000 ± 0.9[a]	49.90 ± 2.7[e]	81.0 ± 0.1[c]	69.90 ± 0.1[d]
p-Coumaric acid	$C_9H_8O_3$	17.25	164.047	2.10 ± 0.5[e]	185.9 ± 0.4[b]	60.20 ± 11.5[d]	195.40 ± 0.4[a]	114.80 ± 0.2[c]
Ferulic acid	$C_{10}H_{10}O_4$	18.25	194.180	2.30 ± 0.2[e]	103.90 ± 0.9[a]	29.7 ± 5.1[d]	91.80 ± 0.2[b]	70.30 ± 0.1[c]
Total phenolic acids				243	484.40	188.50	462.10	304.50

Averages were calculated based on ten replicate samples

Mw molecular weight

Different letters in the same column refer to statistical difference [$p < 0.05$, least significant difference (LSD) test]

*Standard deviation

Table 2. Similarly, boiling improved the chlorogenic acid content in potato cultivars Bintje and Piccolo (Navarre et al., 2010).

In vitro *antioxidant activity*

During the ABTS assay, blue/green $ABTS^+$ is produced, which can be reduced by antioxidants, whilst during the DPPH assay, the antioxidants quench the DPPH radicals by donating hydrogen atoms and decolorising the purple DPPH methanolic solution. Table 3 presents the free radical scavenging activities (DPPH) in raw and cooked pumpkin leaves. Raw leaves and steamed leaves had the highest activities, with the IC_{50}^{DPPH} (18.01 mg mL^{-1}) and (17.40 mg mL^{-1}) respectively, whilst microwaved and stir-fried leaves showed the least antioxidant activities of 47.50 mg mL^{-1} and 48.70 mg mL^{-1} respectively. The high DPPH radical scavenging activity of steamed vegetables compared to the other cooking methods may be because of less antioxidants leaching due to lack of direct contact with water, as shown in Table 2. Cooking decreased the antioxidant capacity (ABTS) of the pumpkin leaves compared to the raw leaves. Among the four adopted cooking methods, steaming showed the significantly ($p < 0.05$) highest antioxidant capacity and similarly steaming increased the FRAP activity in pumpkin leaves (Mashiane, et al., 2021). Steaming also showed the highest DPPH scavenging activity in this study probably due to the presence of higher total phenolic compounds A high DPPH radical scavenging activity was also observed in vegetables with a higher total phenolic content when wild vegetables from Nepal (*Alternanthera sessilis*, *Basella alba*, *Cassia tora*, *Digera muricata*, *Ipomoea aquatica*, *Leucas cephalotes*, *Portulaca oleracea* and *Solanum nigrum)* (Aryal et al., 2019). On the other hand, boiling decreased the antioxidant capacity in spinach and zucchini by between 10 and 30% (Jimenez-Monreal et al., 2009). Conversely, boiled carrot and leeks showed higher antioxidant activity compared to fresh samples (Jimenez-Monreal et al., 2009), and steaming increased the

Table 3 Impact of different household cooking on in vitro antioxidant and glucosidase activities in pumpkin leaves (*Cucurbita moschata*)

Treatments	ABTS (μmoL TEAC g^{-1})	DPPH IC_{50} (mg mL^{-1})	IC_{50} α glucosidase (μg mL^{-1})
Raw	1.52 ± 0.12*[a]	18.01 ± 0.20[c]	20.92 ± 0.15[d]
Boiling	0.76 ± 0.14[c]	32.2 ± 0.30[b]	28.88 ± 0.19[c]
Steaming	0.88 ± 0.10[b]	17.4 ± 0.28[c]	29.81 ± 0.25[b]
Microwaving	0.78 ± 0.21[c]	47.5 ± 0.11[a]	29.69 ± 0.17[b]
Stir-frying	0.66 ± 0.32[d]	48.7 ± 0.16[a]	30.69 ± 0.21[a]
Acarbose			16.98 ± 0.22[e]

Averages were calculated based on ten replicate samples

Different letters in the same column refer to statistical difference [$p < 0.05$, least significant difference (LSD) test]

*Standard deviation

antioxidant properties in green beans (Preti et al., 2017). Antioxidant activity (ABTS) revealed a strong positive correlation with gallic acid (r = 0.77) and protocatechuic acid (r = 0.88), but a strong negative correlation with *p*-coumaric (r = 0.66) and ferulic acid (r = 0.77) (Supplementary Table 1). DPPH activity showed a strong positive correlation with protocatechuic acid (r = 0.88), ellagic acid (r = 0.71) and gallic acid (r = 0.69) (Supplementary Table 1).

In vitro *α-glucosidase enzyme activity*

The effectiveness of the inhibitory effect of raw pumpkin leaves and cooked pumpkin leaves were compared with the commercial antidiabetic agent acarbose (Table 3). Acarbose, the positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} value estimated at 16.98 mg mL^{-1} (Table 3). Raw pumpkin leaves showed the most effective inhibitory effect on α-glucosidase activity with 20.92 mg mL^{-1} compared to the other cooked samples. Therefore, cooking reduced the percentage inhibition of the α-glucosidase enzyme; however, stir-fried pumpkin leaf extract with an IC_{50} value of 30.69 mg mL^{-1} showed the least active of all treatments tested. α-glucosidase is one of the important carbohydrate digestion enzymes found on the brush-border surface membrane of intestinal cells (Anam et al., 2009). α-glucosidase facilitates the production of glucose for intestinal absorption by hydrolysing the disaccharides and oligosaccharides present in the intestine (lumen) (Min and Han, 2014). Inhibition of α- glucosidase, a carbohydrate digestive enzyme, is reportedly one of the most important approaches in managing obesity and diabetes (Assefa et al., 2020).

This study clearly showed that different domestic cooking methods can adversely affect pumpkin leaves phenolic compounds, antioxidant activities and α-glucosidase activity. Cooking methods significantly decreased antinutritive compounds in pumpkin leaves; moreover, results showed strong correlation between phenolic compounds and in vitro α-glucosidase activity. These findings would be useful for consumers when choosing best cooking methods.

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