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The impact of boiling and *in vitro* human digestion of *Solanum nigrum* complex (Black nightshade) on phenolic compounds bioactivity and bioaccessibility

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ABSTRACT

Solanum nigrum complex (Black nightshade) is a wild leafy vegetable with phenolic antioxidant compounds related to the reduction of oxidative stress. Changes in phenolics and bioactivity due to cooking and gastrointestinal digestion of black nightshade were compared to spinach. Predominant compounds of black nightshade were myricetin, quercetin-3-O-robinoside, 3,4-dicaffeoylquinic acid, 3-caffeoylquinic acid, and rutin, which were improved after boiling but reduced after *in vitro* digestion. Phenolics were reduced after digestion of black nightshade and spinach; however, bioactivity was still retained, especially in preventing oxidative stress in Caco-2 cells. Hence, indicating their potential to reduce oxidative stress related diseases of the digestive tract.

1. Introduction

The consumption of foods rich in phenolic compounds has been associated with the promotion of health and reduction of disease risk, (Shahidi, Vamadevan, Oh, & Peng, 2019) through mechanisms such as antioxidant activity, modification of gene expression, and enzyme inhibition (Rosa, Moreno-escamilla, Rodrigo-garcía, & Alvarez-parrilla, 2019). After ingestion, the first site of action for phenolic compounds is the gastrointestinal tract (GIT) which is presented with the highest concentration of phenolics (Villa-Rodriguez, Ifie, Gonzalez-Aguilar, & Roopchand, 2019). Due to their high concentration in the GIT, phenolics' bioactivity has been linked to the protective effects against the development of cancer in the GIT such as colon cancer (Abbas et al., 2017) as well as the prevention of chronic inflammation, such as irritable bowel disease (IBD) (Deiana, Serra, & Corona, 2018).

However, the presence and related bioactivity of phenolic compounds in raw/unprocessed foods does not necessarily reflect the amount and bioactivity after processing and digestion (Gunathilake, Ranaweera, & Rupasinghe, 2018). Heat treatments may induce the structural transformation of polyphenols and/or release from the food matrix, leading to a loss or gain of polyphenols and associated bioactivity (De Santiago, Pereira-Caro, Moreno-Rojas, Cid, & De Peña, 2018). Furthermore, digestion which involves changes in pH and the action of enzymes, may lead to changes in bioaccessibility and bioavailability of phenolic compounds (De Santiago et al., 2018; Murakami, Yamaguchi, Takamura, & Matoba, 2004).

Leafy green vegetables (LGV) are commonly known as good sources of phenolic compounds. In Africa, LGVs consumed include African leafy vegetable (ALVs) and commercial leafy vegetables such as such as kale (Brassica oleracea var. acephala) and spinach (Spinacia oleracea). ALVs comprise of traditional domesticated vegetables that have been part of a traditional production system in Africa for over a number of years as well as vegetables that are indigenous/ biologically originate in Africa (Ambrose-Oji, 2009).

Solanum nigrum complex from Solanaceae family are also known as black nightshade (Särkinen et al., 2018). In South Africa, the most commonly used species are *Solanum americanum*, *Solanum nigrum* and *Solanum retroflexum* (Van Rensburg et al., 2007). In traditional medicine, *S. nigrum* has been used to treat ailments such as diarrhoea, ulcer, inflammation, and jaundice (Campisi et al., 2019). Infusions of

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S. retroflexum have been used for the management of Flu (Mncwango, Mavengahama, Ntuli, & Van Jaarsveld, 2020). Raw/unprocessed black nightshade species have been reported to contain polyphenolics such as chlorogenic acids, apigenin, caffeic acids and gallic acid (Campisi et al., 2019; Neugart, Baldermann, Ngwene, Wesonga, & Schreiner, 2017), as well as anti-radical activity and the ability to inhibit lipid peroxidation (Edith, Souleymane, Souleymane, & Honorine, 2018).

The aim of this study is to determine the effect of cooking/boiling and each step of simulated digestion on black nightshade bioactivity compared with spinach with the purpose to encourage the cultivation, utilization, and the consumption of black nightshade.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were analytical grade and enzymes, α-amylase from *Aspergillus oryzae* (30 U/mg protein), pancreatin from porcine pancreas (8 × USP specification), pepsin from porcine gastric mucosa (250 units/mg solid) were purchased from Sigma-Aldrich Pty. Ltd (Johannesburg, South Africa (SA)). The pBR322 vector DNA (0.5 μg/μL) was purchased from Promega (Madison, WI, USA), while low-density lipoprotein (LDL) (7.6 mg protein/mL) from human plasma, Folin-Ciocalteu reagent, gallic acid, Trolox, and quercetin were also purchased from Sigma-Aldrich Pty. Ltd (Johannesburg, SA).

2.2. Sample preparation

Solanum nigrum Complex specie used in this study was sampled from Tzaneen, Limpopo region, South Africa, February 2018. Professor A. Moteetee, a herbarium curator from the Department of Botany, University of Johannesburg, confirmed the vegetable to be a *Solanum nigrum* complex species, possibly *S. nigrum* or *S. retroflexum*. The two species tend to superficially resemble each other. The spinach was purchased from Woolworths Foods, South Africa. Vegetables were washed, chopped and portioned. A portion of the vegetables was boiled (500 g in 1 l water) for 20 min. Both the raw and boiled vegetables were then freeze-dried at −55 °C for 24 h. The generated samples of black nightshade and spinach were raw vegetable (RV), boiled filtered vegetable (BFV), boiled vegetable -not filtered (BV-NF) and filtrate (F).

2.3. Methanolic extraction

A method according to Moyo, Kayitesi, Mavumengwana, and Madala (2016) was used for methanolic extraction. A mixture containing 2 g powdered leaf material (RV, BV-NF, and BFV) and 20 mL (80% methanol and 1% formic acid) were sonicated for 10 min and centrifuged at 1395 × *g* for 10 min at 4 °C. The supernatant was evaporated to 1 mL by use of a Buchi rotary evaporator, freeze-dried at −55 °C and stored at −20 °C. Phosphate-buffered solution (PBS) was used to reconstitute the powdered extract except for LC-MS the extracts, were reconstituted in methanol:DMSO (1:1).

2.4. Human simulated gastrointestinal digestion

A three-step *in vitro* digestion model was performed according to (Lee, Cha, Kim, Nho, and Pan (2014) with minor modifications that excluded the jejunum, and ileum phases of digestion. For oral digestion, 1 g of the freeze-dried, vegetable (RV, BFV, BV-NF, and F) were mixed and homogenized in 10 mL saline solution consisting of 5 mM KCl, 6 mM $CaCl_2$ and 120 mM NaCl, at pH 5.5. Then, pH was raised to 6.5 after the addition of 1000 units of α- amylase. The mixture was incubated for 5 min in a 37 °C water bath shaking at 95 rpm. Thereafter the samples were subjected to gastric digestion, where the pH was reduced to 2.2 using 0.1 N HCl, then 0.5 mL of a porcine pepsin solution (0.075 g/mL in 0.1 N HCl) was added followed by 15 mL saline solution. The mixture was incubated for 2 h in a 37 °C water bath with shaking at 95 rpm. For duodenal digestion, the mixture from the gastric phase of digestion was mixed with 10 mL of 0.05 M phosphate buffer, pH 7.0 and 3.0 mL of duodenal juice (2 g pancreatin in 60 mL, 240 mL of 50 mM $CaCl_2$, 12.5 g of bile salts, and 0.1 M of $NaHCO_3$, to a final volume of 39.5 mL and the pH was adjusted to 7 with 1 M NaOH). The volume of the duodenal digest was adjusted to 40 mL with phosphate buffer and incubated for 2 h in a 37 °C in a water bath shaking at 200 rpm. The duodenal digested sample was then centrifuged for 15 min at 12,000 × g and 4 °C. The supernatant was used for all analysis and this represents the bio-accessible fraction.

2.5. Determination of phenolic composition

2.5.1. Analytical conditions for UPLC- DAD – QTOF-MS

The analysis was undertaken with high-resolution UPLC-MS according to the method described by Moyo, Serem, Bester, Mavumengwana, and Kayitesi (2020). A Waters Acquity ultra-performance liquid chromatography (UPLC) (Waters, Milford, MA, USA), connected to a Waters Synapt G2 quadrupole time-of-flight (QTOF) mass spectrometer (MS) was used. Data at a mass range of *m/z* 150 to 1500 was collected in resolution mode and MS^E mode. Sodium formate and a lock mass standard (leucine enkephalin) were used to calibrate the instrument to warrant MS accuracy.

2.5.2. Determination of total phenolic content (TPC)

The Folin-Ciocalteu (F-C) method, according to Moyo et al. (2020) was followed to quantify TPC and the absorbance was read at 630 nm using a 96 well microplate plate reader (BMG lab technologies, Offenburg, Germany). Results were reported as mg gallic equivalent GAE/g dry weight (mg GAE/g dw).

2.5.3. Determination of total flavonoid content (TFC)

The aluminium chloride method was performed according to Moyo et al. (2020) to quantify TFC and the absorbance was read at 450 nm using a 96 well microplate plate reader (BMG lab technologies, Offenburg, Germany). Results were reported as mg quercetin equivalent QE/gram dry weight (mg QE/g dw).

2.5.4. Estimation of bioaccessibility

The percentage bioaccessibility (% BA) for TPC (% BA-TPC) and for TFC (% BA-TFC) after simulated gastrointestinal digestion was determined according to Seraglio et al. (2018) using the following equation respectively:

% BA-TPC = PCA /PCB * 100 (i)

% BA-TFC = FCA /FCB * 100 (ii)

PCA is the TPC (mg GAE/g dw) in samples after simulated digestion and PCB is the TPC in undigested samples/before digestion (mg GAE/g dw).

FCA is the TFC (mg QE/g dw) in samples after simulated digestion and FCB is the TFC in undigested samples/before digestion (mg QE/g dw).

2.6. Determination of antioxidant/radical scavenging activity

2.6.1. Trolox equivalence antioxidant capacity (TEAC) or the ABTS assay

The ABTS radical scavenging assay was performed using the method of (Moyo et al. (2020). Briefly, 10 μL sample (1 mg/mL) was mixed with 290 μL of a 0.26 mM ABTS solution and was incubated at 37 °C for 15 min. Absorbance was read at 734 nm, and results reported as μmol Trolox Equivalent (TE)/g dry weight (TE/g dw).

2.6.2. Oxygen radical antioxidant capacity (ORAC) assay

A method according to Moyo et al. (2020) was followed for the ORAC assay procedure. 2, 2′-Azobis-(2-amidinopropane) dihydrochloride (AAPH) was used as peroxyl radical generator and Trolox (0–0.8 mM)

was used as antioxidant standard. A fluorescence plate reader (BMG lab technologies, Offenburg, Germany) set at 37 °C was used to measure fluorescence at an excitation wavelength (Ex) of 485 nm and an emission wavelength (Em) of 520 nm every min for 2 h. The ORAC values were determined by calculating the net area under the decay curves (AUC) and were results reported as μmol TE/g dw.

2.7. Cellular antioxidant activity

2.7.1. 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) assay

The DCFH-DA assay was performed according to Serem (2018). The human colon adenocarcinoma (Caco-2) and mouse fibroblast (L929) cell lines were plated in a 96 well plate at 4×10^3 cells/100 μL and were cultured further in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C for 16 h with 5% CO_2 prior to the measurement of cellular antioxidant activity (CAA). For the measurement of CAA, 50 μL of 75 μM dichlorofluorescein diacetate (DCFH-DA) was added to each well at a final concentration of 25 μM and the plate was incubated at 37 °C for 1 h. The medium was then removed, and the cells were then washed once with PBS, before the addition of 50 μL sample and 50 μL 0.368 mM AAPH. PBS was used as a control. The change in fluorescence was then measured every 2 min for 1 h at an Ex of 485 nm and an Em of 520 nm using a fluorescence plate reader (BMG lab technologies, Offenburg, Germany). The gradient of change in fluorescence was determined and the % oxidative damage (% OD) was calculated using the following equation:

$$\% \text{ OD} = [(\text{Sample} - \text{control PBS}) / (\text{AAPH} + \text{control PBS})] \times 100 \quad \text{(iii)}$$

2.8. Macromolecule protective ability

2.8.1. Copper-mediated human low-density lipoprotein (LDL) oxidation

The copper-mediated LDL oxidation assay was performed according to Moyo et al. (2020). Briefly, sequentially, 100 μL of LDL (100 μg/mL protein), 10 μL sample (1 mg/mL), 10 μL of 0.1 M PBS and 10 μL of 55 μM copper sulphate were added, and the mixture was incubated at 37 ℃ for 16 h. Positive control samples consisted of 100 μL LDL, 20 μL 0.1 M PBS and 10 μL copper sulphate while negative controls consisted of 100 μL LDL and 30 μL 0.1 M PBS. After incubation, the following were sequentially added, 10 μL of 1 mM BHT, 60 μL of a 5 mM Hepes solution, 50 μL of a 10% Trichloroacetic acid (TCA) and 75 μL of a 1% Thiobarbituric acid (TBA) solution. The mixture was incubated in a 60 ℃ water bath for 2 h prior to centrifugation of the samples at 1271 × g for 3 min. The 75 μL supernatant fluorescence was read at an Ex and Em of 544 nm and 590 nm, respectively using a fluorescence plate reader (BMG lab technologies, Offenburg, Germany). Results were reported as μmol Malondialdehyde (MDA)/g dw.

2.8.2. Inhibition of oxidative deoxyribonucleic acid (DNA) damage

Inhibition of DNA oxidative damage was determined according to Kayitesi (2013). AAPH was used as a peroxyl radical generator and 0.1 M PBS was used as a negative control. Briefly, sequentially, 5 μL sample (1 mg/mL), 5 μL of 15 μg/mL PBR322 plasmid and 5 μL of 3.69 mM AAPH were incubated for 1 h at 37 °C. Control samples did not contain AAPH which was replaced with 5 μL of 0.1 M PBS. Supercoiled and linear forms of the plasmid were separated on 1% agarose gel in 1X Tris-acetate EDTA (TAE) buffer with 1% ethidium bromide at 60 V for 2 h. Gel imaging and data analysis was undertaken using an image processing Gel-Pro Analyzer (version 3.0 software – Media Cybernetics, USA) and the percentage supercoiled DNA relative total DNA was determined.

2.9. Statistical analysis

Data were analysed by the use of Statistica software, version 10.0 (Statsoft, Tulsa, USA). Multi-analysis of variance (MANOVA) and Tukey's post hoc tests were used to determine differences between the extraction, and processing methods as well as plant species. A p-value of < 0.05 was used to determine the significant difference, and Pearson's correlation test was utilized to measure the relation between TPC, TFC, and antioxidant activity and a p-value of 1 until 0.8 is considered a very strong correlation and 0.8 to 0.6 is a moderate, hence very strong correlations will be discussed (Akoglu, 2018). Means and standard deviations (±SD) from all experiments are results of at least two experiments performed in triplicate n = 6. SIMCA statistical software version 14.1 (Umetrics, Umea, Sweden) was used for principal component analysis (PCA).

3. Results and discussion

3.1. Phenolic compounds identified in black nightshade by UPLC-DAD-QTOF-MS

Table 1 displays all compounds that were identified in black nightshade extracts using UPLC-DAD-QTOF-MS and includes retention times, the mass-to-charge ratio (*m/z*), molecular formula, diagnostic fragments, and UV maxima. Selected compounds were tentatively identified based on accurate mass and comparison of the elemental compositions to databases such as Metlin and Chemspider. Reference calibrant rutin was used for compounds where no standards were available and to quantify compounds based on the areas of their extracted mass chromatograms.

For this analysis, RV, BFV, and F were analysed to determine the effect of boiling on phenolic metabolites. Further, to determine the effects of *in vitro* digestion on phenolic metabolites, the duodenal digests (final phase of digestion) of RV, BFV, and F were analysed. F represents the fraction often discarded, while BFV is the fraction consumed after boiling. RV is a control representing all polyphenols in the black nightshade.

Different chlorogenic acids (CGAs) were identified. Compound 1 appeared to be a free quinic acid with a [M−H]– ion at *m/z* 191.06 with a fragmentation pattern of *m/z* (127, 111, 93, 85), which is commonly esterified with *trans*-cinnamic acids to form CGAs (Upadhyay & Mohan Rao, 2013). Cinnamate conjugates are formed via the phenylpropanoid pathway and are commonly present in plants as a response to biotic or abiotic stress (Wan, Li, Liu, Chen, & Fan, 2017). Caffeolyquinic acids (CQA) such as 3-caffeoylquinic acid (3CQA), 4-caffeoylquinic acid (4CQA), and 5-caffeoylquinic acid (5CQA) produced a precursor [M−H]-ion at *m/z* 353.09 and fragments 191, 179, 173, 135. Compounds 3,4-dicaffeoylquinic acid (3,4-dCQA) , and 4,5-dicaffeoylquinic acid (4,5-dCQA) produced a parent ion at *m/z* 515.12 and fragments 353, 179 and 173. Similar black nightshade species such as African nightshade (*Solanum scabrum*) also had a high concentration of hydrocinnamic acid derivatives (3-caffeoylquinic acid, 5-caffeoylquinic acid, and 4-caffeoylquinic acid) (Neugart et al., 2017).

Different subclasses of flavonoids were detected. The flavanol catechin, was identified with a parent ion of 289.07 and fragments of 245, 205, 203, 123, and 109. Flavonol myricetin, which is also known as hydroxyquercetin due to its structural resemblance to quercetin (Semwal, Semwal, Combrinck, & Viljoen, 2012), was also tentatively identified at precursor [M−H]-ion *m/z* 315 and fragments 255, 375. Further, flavonols quercetin-3-O-robinobioside (Uvmax 257 & 353) and rutin (Uvmax 353) produced [M−H]–ion at *m/z* 609.14 and fragments 300, 301, 271, and 255. Previously, rutin, quercetin, and catechin have been identified in black nightshade (Chang et al., 2017; Yang et al., 2010).

3.2. Effect of boiling and in vitro digestion on identified compounds

Heat applied to vegetables during processing can cause structural and chemical changes to phenolic compounds, negatively or positively, depending on the cooking technique and food matrix (De Santiago et al., 2018). Therefore, in this study, the principal component analysis (PCA)

Table 1
Compounds identified in black nightshade raw, boiled, and digested samples by UPLC-QTOF-MS.

Retention time	Precursor ion $[M-H]^-$	Formula $[M-H]^-$	Diagnostic fragments	UV max	Compound name	Effects of boiling			Effects of *in vitro* digestion		
						RV	BFV	F	RV	BFV	F
1.71	191.06	$C_7H_{12}O_6$	127, 111, 93, 85	210	Quinic acid	$0,11^{b}$	$0,14^{b}$	$4,12^{d}$	$0,53^{c}$	$0,19^{b}$	0^{a}
9.86	353.09	$C_{16}H_{17}O_9$	191, 179, 173, 135	326	3-Caffeoylquinic acid	$19,23^{a}$	$1047,07^{c}$	$2346,58^{d}$	0^{a}	$108,51^{b}$	$275,80^{b}$
11.36	289.0705	$C_{15}H_{13}O_6$	245, 205, 203, 123, 109	278	Catechin	0.30^{a}	14.15^{b}	9.91^{ab}	5.62^{ab}	1.86^{a}	9.32^{ab}
11.42	353.09	$C_{16}H_{17}O_9$	191, 179,173,135	326	4-Caffeoylquinic acid	0.56^{a}	55.41^{c}	284.09^{d}	0^{a}	8.64^{b}	32.10^{c}
12.41	353.09	$C_{16}H_{17}O_9$	191, 179, 173, 135	326	5-Caffeoylquinic acid	18.14^{a}	1276.4^{e}	928.03^{d}	0^{a}	139.82^{b}	329.94^{c}
16.57	317.237	$C_{15}H_9O_8$	315	255, 375	Myricitin	18.80^{c}	30.33^{c}	8.27^{b}	21.12^{c}	0.36^{a}	0^{a}
16.87	609.1451	$C_{27}H_{29}O_{16}$	300, 301, 271, 255	257, 353	Quercetin-3-O-robinobioside	1.47^{ab}	26.87^{c}	3.55^{ab}	1.72^{ab}	5.68^{b}	0^{a}
17.14	609.1477	$C_{27}H_{29}O_{16}$	300, 301, 271, 255	353	Rutin	4.18^{a}	89.31^{c}	166.80^{d}	0.84^{a}	6.11^{a}	25.38^{b}
17.64	463.0857	$C_{21}H_{19}O_{12}$	300, 301, 271, 255	348	Quercetin-3-O-glucoside	0^{a}	117.74^{c}	66.9^{bc}	0^{a}	38.87^{bc}	261.05^{d}
19.01	515.12	$C_{25}H_{23}O_{12}$	353, 179, 173	325	3,4-Dicaffeoylquinic acid	30.76^{ab}	277.87^{c}	363.40^{d}	0^{a}	12.33^{a}	87.84^{b}
19.69	515.12	$C_{25}H_{23}O_{12}$	353, 179, 173	325	3,5-Dicaffeoylquinic acid	0^{a}	150.21^{b}	242.85^{c}	0^{a}	0^{a}	22.22^{a}
20.84	515.12	$C_{25}H_{23}O_{12}$	353, 179, 173	325	4,5-Dicaffeoylquinic acid	45.30^{a}	124.50^{b}	47.56^{a}	54.95^{a}	5.67^{a}	129.87^{b}
22.35	491	$C_{23}H_{23}O_{12}$	285, 151, 135	307	Unknown-kaempferol	1.26^{a}	16.29^{d}	2.84^{b}	9.75^{c}	0.72^{a}	0.82^{a}
23.86	301.0336	$C_{15}H_9O_7$	151, 179	256	Quercetin	3.31^{a}	2.59^{a}	20.00^{c}	0^{a}	0.72^{a}	11.95^{b}

*Phenolic compound concentration expressed as μg/g extract. Raw vegetable (RV), boiled, filtered vegetable (BFV), F- filtrate. All data reported as means ± SD (n = 2). Alphabets in superscripts within a raw show significant differences at ($P < 0.05$).

model was used to depict the influence occurring due to boiling and extraction (methanolic and *in vitro* digestion) methods. As shown in Fig. 1, the first two principal components (PCs) explained 75.4% of the total variation. PC-1 accounted for 42.8% of the variation in the X matrix (R^2Y, cum) with the predictive ability of 88.7% (Q^2, cum) while PC-2 explained 22.6% of the variation.

The PC-1 positive axis (Fig. 1) grouped the methanolic BFV (brown) and F (red) samples as both samples were dominated by 3,4-dCQA, 3,5-dCQA, 3-CQA, 4-CQA, 5-CQA, and rutin as also shown in Table 1. The PC-1 negative axis grouped the methanolic RV (green), with the digested RV (yellow) BFV (blue) and F (light blue) vegetable residues together as these samples possessed lesser concentrations of the compounds found in PC-1 positive axis. As such, it is observed that the boiling of the vegetable could have increased the extractability of these phenolics by the degradation of cell walls and subcellular structures. Phenolic compounds are highly soluble in water (Murador, Braga, Da Cunha, & De Rosso, 2018); and all polyphenols were present in the filtrate. This prompted the inclusion of the boiled vegetable not filtered (BV-NF) samples in subsequent analysis. The filtrate contained higher concentrations of CGAs than flavonoids, hence, depicting a greater susceptibility of CGAs to leaching than flavonoids.

The separation of methanolic BFV (brown) from the digested BFV (blue) in the PCA model (Fig. 1) shows a significant effect of digestion on the presence of phenolics. Table 1 shows a significant ($p < 0.05$) decrease in compounds such as 3-CQA, catechin, 4-CQA, 5-CQA, myricetin, quercetin-3-O-robinoside, 3,4-dCQA, 3,5-dCQA, 4.5-dCQA and rutin in BFV after digestion. However, digestion did not cause significant ($p < 0.05$) changes in compounds found in raw vegetable samples as depicted in PCA model and Table 1. Hence, suggesting the effect of vegetable material during digestion. Digestion involves the breakdown of insoluble food molecules, such as carbohydrates and proteins by pH changes and digestive enzymes which yield smaller soluble molecules

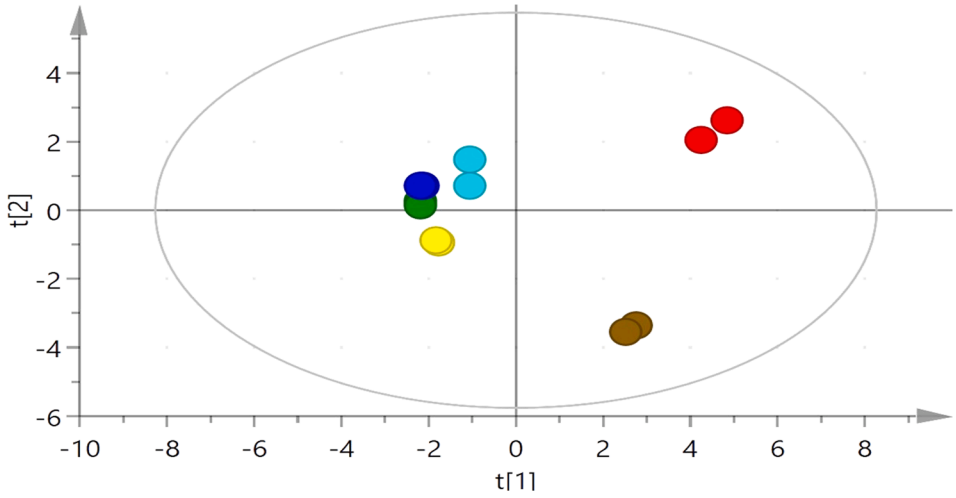

Fig. 1. Principal component analysis (PCA) multivariate projection score scatter plot of metabolites from black nightshade. Samples - filtrate F-water extract (red) and boiled filtered vegetable BFV- methanol extract (brown), raw vegetable RV - methanol extract (green), digests RV (yellow), digests BFV (blue), and digests F (light blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Shahidi & Peng, 2019), or the hydrolysis of some phenolic metabolites, which can result in changes in chemical structure, solubility, and molecular weight (Seraglio et al., 2018). The observed effect is usually a combination of these processes.

Although our study shows a decrease in some phenolics for the boiled vegetable sample and no significant changes for the raw vegetable sample after duodenal digestion, phenolics extraction could be enhanced by the action of gut microbiota in the large intestine (Cueva, Silva, Pinillos, Bartolomé, & Moreno-Arribas, 2020).

3.3. Effect of boiling and in vitro digestion on TPC and TFC

The effects of boiling on TPC and TFC of black nightshade and spinach was determined and compared. For the methanolic extracts, the TPC and TFC of raw spinach were significantly ($p < 0.05$) greater than levels found in raw black nightshade (Table 2). In other studies, a higher TPC (mg GAE/g dw) of 300.00 ± 5.70 and lower TFC (mg QE/g dw) of 54.60 ± 8.30 was reported by Edith et al. (2018) for black nightshade and further lower TPC values in the range 2.29 mg GAE/g dw – 71.67 mg/g dw have been reported by Amin, Norazaidah, and Hainida (2006); Howard, Pandjaitan, Morelock, and Gil (2002); Zhou and Yu (2006) for spinach. Variation in results could be attributed to the different methods of extraction and solvents used, while geographical location, rainfall, soil conditions, and stress can influence the polyphenol content of plants.

Table 2
Total phenolic content (TPC), total flavonoid content (TFC) and % bioaccessibility (% BA) of TPC, TFC of black nightshade and spinach.

Samples	TPC mg GAE/g dw	TFC mg QE/g dw	TPC% BA	TFC% BA
Black nightshade Methanolic extract				
RV	199.01 ± 2.6 ^{g}	73.86 ± 2.6^{i}	–	–
BFV	193.46 ± 10 ^{g}	67.03 ± 0.7^{ghi}	–	–
BV-NF	207.73 ± 0.4 ^{g}	62.56 ± 1.7 ^{fg}	–	–
Oral digestion				
RV	124.53 ± 16^{def}	111.10 ± 3.9 ^{k}	62.57	150.42
BFV	92.13 ± 7.2^{bcd}	70.20 ± 3.3^{hi}	47.62	104.73
BV-NF	92.97 ± 6.9^{bcde}	90.42 ± 5.6^{j}	44.75	144.53
Gastric digestion				
RV	102.43 ± 15^{cdef}	58.96 ± 2.0^{f}	51.47	79.83
BFV	129.82 ± 15^{f}	71.23 ± 6.2^{hi}	67.10	106.26
BV-NF	57.55 ± 3.4^{ab}	45.68 ± 5.6^{e}	27.70	73.02
Duodenal digestion				
RV	104.32 ± 7.0^{cdef}	66.64 ± 3.4^{gh}	52.42	70.97
BFV	99.50 ± 2.4^{cdef}	48.83 ± 5.6^{e}	51.43	72.84
BV-NF	35.67 ± 8^{a}	19.63 ± 1.8^{ab}	17.17	31.38
Digestion effect on				
RV	Reduced	Reduced	–	–
BFV	Reduced	Reduced	–	–
BV-NF	Reduced	Reduced	–	–
Spinach Methanol extract				
RV	349.38 ± 7.8^{j}	133.09 ± 2.6 ^{l}	–	–
BFV	279.10 ± 1.7^{hi}	93.41 ± 0.89^{j}	–	–
BV-NF	289.78 ± 4.4^{i}	93.82 ± 2.7^{j}	–	–
Oral digestion				
RV	248.58 ± 4.8 ^{h}	69.14 ± 3.2^{ghi}	71.14	51.94
BFV	115.18 ± 5.2^{def}	23.76 ± 1.4^{bc}	41.27	25.44
BV-NF	128.15 ± 3.9^{ef}	42.11 ± 2.1^{e}	44.22	44.88
Gastric digestion				
RV	260.03 ± 2.4^{hi}	48.83 ± 4.7^{e}	74.43	36.69
BFV	201.68 ± 2.9 ^{g}	33.76 ± 0.18^{d}	72.26	36.14
BV-NF	401.21 ± 2.9^{j}	33.17 ± 4.2 ^{l}	138.45	35.35
Duodenal digestion				
RV	124.25 ± 8.9^{def}	29.06 ± 3.13 ^{cd}	35.56	21.83
BFV	93.88 ± 6.0^{cde}	16.44 ± 2.1^{a}	33.64	17.60
BV-NF	69.01 ± 2.7^{abc}	17.37 ± 1.3^{ab}	23.81	18.51
Digestion effect on				
RV	Reduced	Reduced	–	–
BFV	Reduced	Reduced	–	–
BV-NF	Reduced	Reduced	–	–

Results expressed as dry matter weight (dw), GAE - gallic acid equivalent, QE - quercetin equivalent, TPC - total phenolic contents, TFC - total flavonoid content. All data reported as means ± SD (n = 6) of 2 experiments done in triplicate. Alphabets in superscripts within a column show significant differences at ($p < 0.05$). Raw vegetable (RV), boiled filtered vegetable (BFV), boiled vegetable – not filtered (BV-NF)

The process of boiling did not significantly alter the TPC of black nightshade methanol extracts. In contrast, the TFC of BV-NF was significantly ($p < 0.05$) reduced at 15.07%. For spinach, TPC was reduced although not significant while TFC was significantly ($p < 0.05$) reduced for BFV and BFV-NF, suggesting flavonoid instability due to thermal degradation (Subudhi & Bhoi, 2014). Although the loss of TFC was 30.07% for spinach, TFC levels were still greater than that of black nightshade. Roy, Takenaka, Isobe, and Tsushida (2007) noted a 50.8% loss of TPC in spinach due to boiling at 100 ℃ for 30 min. In other studies, increases in TPC after boiling were reported for leafy vegetables such as Tanner's cassia (*Cassia auriculata* L.) boiled for 5 min, and Jute mallow (*Corchorus olitorius* L.) boiled for 30 min (Gunathilake et al., 2018; Mavhungu, 2011).

The extractability of polyphenols from solid matrices of leafy vegetables is a requirement for bioaccessibility and bioavailability (Palafox-Carlos, Ayala-Zavala, & González-Aguilar, 2011). Bioaccessible (BA) polyphenols can protect the mucosa of the GIT against oxidative stress while bioavailability has systemic antioxidant benefits. For RV samples, % BA-TPC of black nightshade did not change significantly during oral, gastric, and duodenal phases of digestion. For spinach, the % BA-TPC for RV was reduced for the oral, gastric, and duodenal phases of digestion. This indicates that the polyphenols in the RV are susceptible to the effects of pH and digestive enzymes during digestion (Table 2).

For boiled black nightshade, a decrease in % BA for BFV and BFV-NF occurred, with a greater decrease observed for BFV-NF and possibly indicates the susceptibility of polyphenols in the filtrate to degradation. For the boiled spinach, an increased % BA for the gastric phase was due to the increased extractability of polyphenols at low pH, while a lower % BA during the duodenal phase was caused by flavonoid susceptibility to degradation in alkaline conditions.

Gastric enzymatic action and acid hydrolysis of phenolics bound to dietary fibre or proteins increase % BA-TPC during gastric digestion (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2014). For duodenal digestion, the more hydroxylated a phenolic molecule is, the lower the stability it would possess under alkaline conditions (Wang & Zhao, 2016). For example, flavonoids such as fistein and quercetin degrade at neutral or slightly alkaline pH (Wang & Zhao, 2016). Low bioaccessibility of polyphenols at the duodenal phase of digestion could also be caused by the formation of water-soluble mixed micelles during the transition from low gastric to high duodenal pH in the presence of intestinal enzymes and macromolecule content of digested plant material (Caicedo-Lopez, Luzardo-Ocampo, Cuellar-Nuñez, Campos-Vega, Mendoza, & Loarca-Piña, 2019).

3.4. Effect of boiling and in vitro digestion on antioxidant activity

3.4.1. Radical scavenging activity and redox potential

The importance of antioxidants relates to their ability to reduce the risk of diseases such as hypertension, CVD, cancers, diabetes, and inflammation by metal chelation, reduction potential, radical scavenging activities and upregulation of the endogenous antioxidant defence system of the body (Kumar, Krishna Chaitanya & Preedy, 2018; Shahidi, Zhong & Chandrasekara, 2012). Different antioxidant assays can be used to evaluate several aspects of antioxidant activity, where the TEAC assay evaluates the ability of antioxidants such as polyphenols to donate electrons, and the ORAC assay, donation of a hydrogen atom.

The effects of boiling on the antioxidant activity of all black nightshade and spinach samples was determined by the TEAC and ORAC assays (Table 3). Firstly, the effects of boiling and then the effect of digestion was determined.

For the TEAC assay, boiling did not significantly alter the radical scavenging activities of black nightshade and spinach methanolic extracts. Likewise, boiling of curly kale (*Brassica oleracea* var. *acephala*) leaves did not significantly alter antioxidant activity from 119.01 ± 0.41 μM TE/g dw to 119.72 ± 1.19 μM TE/g dw (Kapusta-Duch, Kusznierewicz, Leszczyńska, & Borczak, 2016).

The hydroxyl scavenging activity as measured by the ORAC assay revealed significant ($p < 0.05$) increases for the black nightshade and spinach methanolic extracts after boiling. In other studies, the % hydroxyl scavenging activity of *Solanum melongena* also increased from 50.62 ± 1.78% to 54.44 ± 0.46% after boiling for 20 min (Nwozo, Oso, & Oyinloye, 2015). Increases in antioxidant activity after boiling reported regarding the ORAC assay, could be related to the degradation of cell walls and the release of molecules with hydroxyl scavenging activity from the cellular structures.

The correlations between different assays were determined. For black nightshade, poor correlations i.e. below 0.600 were obtained between TPC and TFC and each antioxidant assays, indicating that other non-polyphenols and/or flavonoids are contributing to antioxidant activity. The presence of other antioxidant molecules or other interfering molecules (Álvarez, Araya, Navarro-lisboa, & Dicastillo, 2016), may also skew correlations. For example, ascorbic acid, known to be present in spinach, will not contribute to TPC or TFC but will contribute significantly to antioxidant activity measured with the ORAC assay.

The bioactivity of antioxidants after digestion may differ quantitatively and qualitatively from those produced by chemical extraction (Serrano, Goñi, & Saura-Calixto, 2007). For all black nightshade and spinach digested fractions, antioxidant activity measured with the TEAC assay was significantly ($p < 0.05$) less than the methanolic extracts. In contrast, activity measured with the ORAC assay was increased in the RV, unchanged in BFV and reduced in BV-NF.

Differences in measured antioxidant effects are related to the effect being measured and are a function of the type and polyphenol concentration. The degradation, transformation, and hydrolysis reactions of phenolic compounds during digestion may yield lower antioxidant activity. Factors such as the chemical structure of the food matrix in which the polyphenols are found, may affect the antioxidant capacities of digested vegetables (Caicedo-Lopez, Luzardo-Ocampo, Cuellar-Nuñez, Campos-Vega, Mendoza, & Loarca-Piña, 2018). Furthermore, possible synergistic interactions depending on the phenolic profile of the extract will also affect the antioxidant activity (Caicedo-Lopez et al., 2018).

Glycosylated and esterified phenolics could be hydrolysed by acid conditions of the gastric phase (Mawalagedera et al., 2016), thus increasing the availability of –OH groups as was found by (Gonçalves, Moreira, Andrade, Valentão, & Romano, 2018), where gastric digestion caused an increase in TPC and ferric reducing antioxidant power (FRAP) activity of Bermuda buttercup (*Oxalis pes-caprae*).

Dietary fibre may act as an entrapping matrix that restricts the diffusion of phenolics released during gastric phase digestion, hence, restricting their extraction into the digestion fluid (Yang, Jayaprakasha, & Patil, 2018). Further, phenolics highly interact with pancreatin bile salts and digestive enzymes to form insoluble complexes, hence, a decrease in their original values and bioactivity (Spínola, Llorent-Martínez, & Castilho, 2018). Polyphenols including quercetin and fistein are sensitive to the effects of pH (Wang & Zhao, 2016). The pH of the reaction environment contributes to the stability and free radical scavenging activity of polyphenol compounds and is a function of the various dissociable –OH groups in their chemical structure (Ghosh, Chakraborty, & Raychaudhuri, 2015). Polyphenols may have the ability to autooxidise under neutral to alkaline conditions of the small intestine (Bayliak, Burdyliuk, & Lushchak, 2016). These effects may contribute to the reduced activity for BFV of black nightshade and spinach after

Table 3
Radical scavenging activity and cellular antioxidant activity of black nightshade and spinach.

Samples	TEAC μM TE/g dw	ORAC μM TE/g dw	%OD DCFH-DA$_{L929}$	%OD DCFH-DA$_{Caco-2}$
Black nightshade				
Methanolic extract			100^{e*}	100 ^{h*}
RV	650.95 ± 5.4^{hij}	299.30 ± 5.0^{a}	−8.85 ± 12^{ab}	−3.21 ± 13^{abcde}
BFV	654.13 ± 5.9^{hij}	741.12 ± 8.5^{cdef}	−18.10 ± 3.8^{a}	−2.57 ± 3.2^{abcde}
BV-NF	576.00 ± 8.8^{ghi}	864.35 ± 3.0^{ef}	−15.81 ± 6.1^{ab}	−7.51 ± 0.6^{abc}
Oral digestion				
RV	403.14 ± 5.6^{cdef}	788.37 ± 2.4^{cdef}	272.46 ± 5.6^{f}	242.47 ± 1.8^{i}
BFV	355.06 ± 3.3 ^{cd}	769.88 ± 3.3^{cdef}	−0.171 ± 1.5^{abc}	6.62 ± 0.1^{cdefg}
BV-NF	340.21 ± 6.9^{c}	326.85 ± 1.3^{ab}	20.50 ± 5.1^{bc}	1.27 ± 12^{abcdefg}
Gastric digestion				
RV	409.50 ± 4.4^{cdef}	862.21 ± 9.8^{ef}	31.60 ± 3.6 ^{cd}	0.87 ± 1.6^{abcdef}
BFV	501.77 ± 1.8^{efg}	841.80 ± 1.7^{def}	65.02 ± 5.5^{de}	5.70 ± 5.8^{cdefg}
BV-NF	313.70 ± 5.2^{bc}	324.32 ± 1.6^{ab}	9.245 ± 0.1^{abc}	−4.91 ± 7.4^{abcd}
Duodenal digestion				
RV	379.45 ± 3.4^{cde}	620.80 ± 4.5^{bcde}	−11.87 ± 0.7^{ab}	13.76 ± 3.0 ^{fg}
BFV	314.41 ± 1.9^{bc}	608.70 ± 6.1^{bcde}	−6.69 ± 0.8^{ab}	3.24 ± 11^{bcdefg}
BV-NF	158.87 ± 10^{a}	330.16 ± 8.9^{ab}	−1.96 ± 6.2^{abc}	−2.48 ± 5.4^{abcde}
Digestion effect on				
RV	Reduced	Increased	Unchanged	Increased
BFV	Reduced	Unchanged	Unchanged	Unchanged
BV-NF	Reduced	Reduced	Unchanged	Unchanged
Spinach				
Methanol extract				
RV	1090.99 ± 1.4 ^{l}	756.20 ± 1.5^{cdef}	−21.52 ± 4.7^{a}	−5.39 ± 2.7^{abcd}
BFV	1021.96 ± 2.4 ^{l}	1223.21 ± 9.7^{gh}	−20.33 ± 4.1^{a}	−13.39 ± 6.8^{a}
BV-NF	1084.84 ± 1.5 ^{l}	1181.94 ± 2.5^{gh}	−20.57 ± 4.8^{a}	−10.71 ± 1.0^{ab}
Oral digestion				
RV	712.53 ± 9.8^{ij}	637.67 ± 3.7 ^{h}	102.05 ± 1.0^{e}	16.49 ± 0.8 ^{g}
BFV	495.93 ± 9.3^{defg}	1363.33 ± 1.7 ^{h}	−6.86 ± 3.7^{ab}	10.19 ± 1.1^{defg}
BV-NF	370.37 ± 3.3^{cde}	540.65 ± 4.3^{abc}	−7.22 ± 4.1^{ab}	6.64 ± 0.3^{cdefg}
Gastric digestion				
RV	857.14 ± 7.0 ^{k}	1681.07 ± 5.2^{i}	−0.006 ± 7.5^{abc}	−0.83 ± 0.2^{abcdef}
BFV	748.83 ± 5.8^{ijk}	1179.40 ± 7.2^{gh}	−20.59 ± 5.1^{a}	−0.053 ± 0.04^{abcdef}
BV-NF	1090.99 ± 9.3 ^{l}	546.13 ± 4.6^{abcd}	−10.21 ± 6.1^{ab}	10.74 ± 1.9^{efg}
Duodenal digestion				
RV	534.01 ± 6.6^{fgh}	1000.71 ± 1.2 ^{fg}	−3.19 ± 0.02^{abc}	−10.90 ± 5.1^{ab}
BFV	523.30 ± 5.9^{fgh}	1004.29 ± 7.8 ^{fg}	−3.09 ± 0.8^{abc}	−5.26 ± 2.7^{cdefg}
BV-NF	172.21 ± 1.5^{ab}	563.79 ± 2.2^{abcd}	−5.61 ± 1.4^{abc}	−7.16 ± 1.1^{abc}
Digestion effect on				

(*continued on next page*)

Table 3 (*continued*)

Samples	TEAC μM TE/g dw	ORAC μM TE/g dw	%OD DCFH-DA$_{L929}$	%OD DCFH-DA$_{Caco-2}$
RV	Reduced	Increased	Unchanged	Unchanged
BFV	Reduced	Unchanged	Unchanged	Reduced
BV-NF	Reduced	Reduced	Unchanged	Unchanged

Results expressed as dry matter weight (dw), μM TE - microMolar Trolox equivalent, OD - oxidative damage, DCFH-DA – 2′,7′-dichlorodihydrofluorescein diacetate, L929 - mouse fibroblast, Caco-2 - human epithelial colorectal adenocarcinoma, TEAC- Trolox equivalence antioxidant capacity, ORAC - oxygen radical antioxidant capacity. All data reported as means ± SD (n = 6) of 2 experiments done in triplicate. Alphabets in superscripts within a column show significant differences at ($p < 0.05$). Raw vegetable (RV), boiled filtered vegetable (BFV), boiled vegetable – not filtered (BV-NF). *Control cells (AAPH + cells).

digestion measured with the TEAC assay.

3.5. Cellular antioxidant activity

In addition to chemical antioxidant assays, the antioxidant activity can be determined in mammalian cells, which takes into consideration the effects of conditions such as temperature, pH as well as the sum of cellular effects that include bioavailability, distribution metabolism, and excretion (Gutiérrez-Grijalva, Antunes-Ricardo, Acosta-Estrada, Gutiérrez-Uribe, & Basilio Heredia, 2019). In the GIT, mucosa epithelial cells are often the initial site of oxidative stress. Therefore, the measurement of cellular antioxidant activity, against AAPH peroxyl radical-induced oxidation of non-fluorescent 2′,7′-dichlorodihydrofluorescein (DCFH) to a fluorescent 2′,7′-dichlorofluorescein (DCF) in the L929 and Caco-2 cell lines representing fibroblasts and physiologically relevant colonic cell line respectively, was determined.

In this study, the DCFH-DA assay results were expressed as a percentage (%) in which the control (oxidant, AAPH) causes 100% cellular oxidative damage. Values < 100%, if statistically significant, indicates an antioxidant protective effect while values > 100% indicates oxidative damage (OD) or a pro-oxidant effect.

In the L929 cell line, all black nightshade and spinach extract protected both cell types from oxidative damage with the exception of the RV (oral) samples from black nightshade and spinach that presented 272.46% ± 5.6 and 102.05% ± 1.0 OD respectively, indicating that cooking may reduce oxidative effects or that with cooking the oxidative levels are reduced so that under these experimental conditions a protective effect is observed rather than an oxidative damage effect as compared to the control. For the Caco-2 cell line, a high % OD was observed for black nightshade oral RV extracts at 242.47 ± 1.8% while all other samples protected the Caco-2 cell line. After digestion, for all black nightshade and spinach fractions, the % OD for the L929 cell line remained unchanged as compared to the methanolic extracts. However, with the Caco-2 cell line, % OD increased after the duodenal digestion of black nightshade whereas BFV and BV-NF extracts' % OD remain unchanged, hence, indicating a loss of protective effect against oxidative damage after complete digestion of RV rather than the cooked samples. For spinach, the RV and BV-NF extracts' % OD of the Caco-2 cell line was unchanged while it improved (reduced) for the BFV extracts.

Antioxidants have different mechanisms of action; chemical assays measure the direct reaction of antioxidant compounds with reactive species while cell-based assays also take into consideration cellular uptake, metabolism, the inhibition of oxidant enzymes as well as interaction with redox signalling pathways (Camilo López-Alarcón & Denicola, 2013; Kellett, Greenspan, & Pegg, 2018). A strong negative correlation between assays that measure content and antioxidant activity and % OD measured in the L929 and Caco-2 cell lines indicate a beneficial effect possibly related to the effective adsorption of antioxidant compounds to the cell membrane under gastrointestinal conditions (Chen, Ma, Fu, & Yan, 2017).

For black nightshade, a strong negative correlation was found between % OD and TFC as well as antioxidant activity measured with the TEAC assay indicating the flavonoids with antioxidant activity contribute to cellular antioxidant activity evaluated in the L929 cell line. Although a poorer correlation was found, a negative correlation was found for all the parameters with duodenal digestion. In contrast in the Caco-2 cell line, no such correlation was found. For spinach, a negative correlation was obtained between ORAC and % OD $_{Caco-2}$ at −0.943 for gastric digestion signify prevention of oxidative damage through hydroxyl scavenging activity, although it is possible that other non-phenolic compounds present in plants such as ascorbic acid, carotenoids, glutathione, and proline (Kasote, Katyare, Hegde, & Bae, 2015) could have contributed to the protective ability of black nightshade and spinach against oxidative damage. Therefore, it may be recommended that in the future, dosage-dependent studies on extracts and digests that provide the greatest degree of protection can be identified.

3.6. Macromolecule protective ability

3.6.1. Copper-mediated human LDL oxidation

Low-density lipoproteins function as transporters of cholesterol in human blood plasma, and their oxidation by metals such as Cu^{2+} contributes to the development of atherogenesis (Amarowicz, 2016).

Antioxidants such as Trolox, a vitamin E analogue can reduce Cu^{2+} induced oxidative damage as shown in Fig. 2 where 1 mM Trolox prevents LDL oxidation. The black nightshade methanolic extracts of RV and BV-NF significantly ($p < 0.05$) inhibited LDL oxidation, whereas no effect was observed for BFV, indicating that protective molecules leached from the plant matrix and as part of the filtrate protects LDL against oxidation Fig. 2. No inhibition was observed for any of the spinach methanolic extracts. All digested extracts from black nightshade and spinach also did not inhibit the oxidation of LDL. Flavonoids can inhibit LDL oxidation by reducing the copper-binding site on the LDL molecule, hence, preventing the ion from oxidizing the LDL; also, flavonoids can act by neutralizing radicals such as aldehydes during oxidation (Dianita & Jantan, 2019).

3.6.2. Inhibition of AAPH- induced oxidative DNA damage

DNA damage and inefficient repair caused by reactive oxygen/nitrogen species are associated with cellular injury and the induction of carcinogenesis (Chandrasekara, Daugelaite, & Shahidi, 2018; Tiwari & Mishra, 2017). Effects include free radical-induced DNA strand breaks, DNA -protein cross-links, base modification of free sites, and abnormal chromosomal arrangements (Chandrasekara et al., 2018).

The effects of black nightshade and spinach on DNA in the presence of AAPH was determined (Fig. 3). The % supercoiled DNA recovered for methanolic extracts and digests of black nightshade incubated with AAPH, was significantly ($p < 0.05$) greater than the % supercoiled DNA recovered for corresponding samples of spinach.

Although spinach exhibited higher contents of phenolics and flavonoids than black nightshade, its inhibitory activity towards DNA damage was lower. Similar to these results, Yujing, Zhaojie, Hu, Yongliang, and Liping (2018) noted that while TPC of plant extracts may be high, this does not necessarily translate into increased protection against DNA damage and consequently other factors, such as the number of hydroxyl groups of the quantified phenolics, may influence the bioactivity.

For the methanolic and gastric extracts of black nightshade, the single electron transfer mechanism evaluated with the TEAC assay was largely responsible for the protection of DNA due to a strong positive correlation of 0.829 with the TEAC. Further, hydroxyl donating property (ORAC) of oral and duodenal extracts of black nightshade strongly contributed to the protection of DNA damage with a coefficient (r) of 0.714 and 0.829, respectively with polyphenols including flavonoids contributing to this activity indicated by the strong correlation of 0.886 and 0.943 for TPC and TFC, respectively. The TPC and TFC of spinach are greater than black nightshade, and consequently, a higher

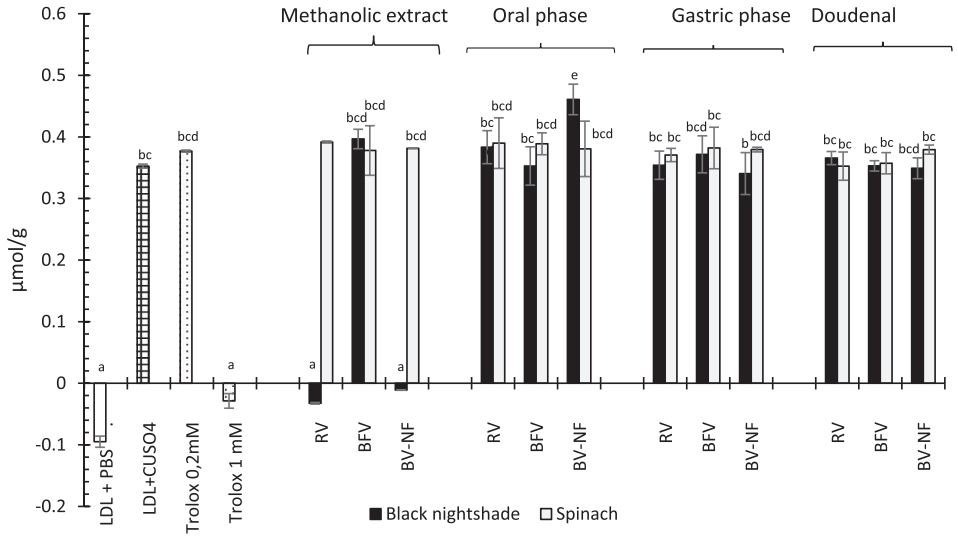

Fig. 2. Inhibition of copper-mediated human LDL oxidation by black nightshade and spinach extracts. Controls: LDL and phosphate-buffered solution (LDL + PBS), LDL and Cu^{2+} (LDL + Cu^{2+}) and antioxidant controls, LDL + Cu^{2} with 0.2 and 1 mM Trolox (Trolox 0,2 mM and 1 mM). All samples contain LDL, Cu^{2+} and 10 µL sample (1 mg/mL). Samples were raw vegetable RV, boiled filtered vegetable BFV, boiled vegetable – not filtered BV-NF.

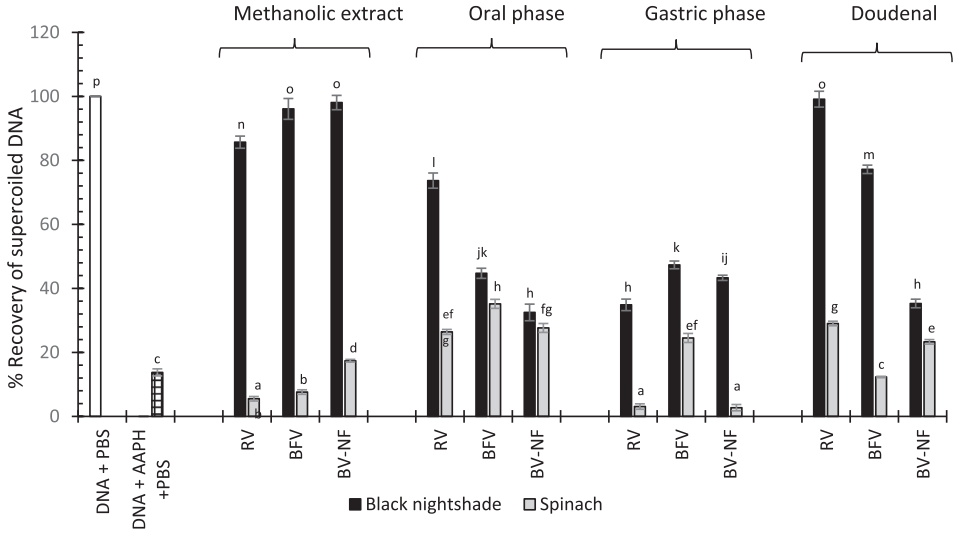

Fig. 3. % Recovery of supercoiled DNA for methanolic extracts and digests of black nightshade (black bars) and spinach (grey bars). Controls: DNA + PBS and DNA + AAPH (1 mg/ml) + PBS. Samples are methanolic extracts and digests of raw vegetable (RV), boiled filtered vegetable (BFV), boiled vegetable – not filtered (BV-NF).

concentration of polyphenols may contribute to a pro-oxidant effect.

4. Conclusion

The process of boiling may reduce or increase the phenolic content of the studied vegetables. Spinach exhibited a higher TPC and TFC than black nightshade before and after digestion of raw and boiled vegetable. The bioaccessibility of phenolic compounds in both black nightshade and spinach decreased after *in vitro* digestion. However, sufficient bioactivity was still retained after digestion with black nightshade showing better protective properties towards plasmid DNA oxidative damage than spinach. Further, oxidative stress was prevented by both black nightshade and spinach in the intestinal environment, therefore, indicating their potential to prevent/reduce the development of cancer in the GIT, such as colon cancer as well as the prevention of chronic inflammation, such as irritable bowel disease.

CRediT authorship contribution statement

S.M. Moyo: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization. **J.C. Serem:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Visualization. **M.J. Bester:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision. **V. Mavumengwana:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision. **E. Kayitesi:** Conceptualization, Methodology, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abbas, M., Saeed, F., Anjum, F. M., Afzaal, M., Tufail, T., Bashir, M. S., … Suleria, H. A. R. (2017). Natural polyphenols: An overview. *International Journal of Food Properties, 20*(8), 1689–1699. https://doi.org/10.1080/10942912.2016.1220393

Akoglu, H. (2018). User's guide to correlation coefficients. *Turkish Journal of Emergency Medicine, 18*(3), 91–93. https://doi.org/10.1016/j.tjem.2018.08.001

Álvarez, R., Araya, H., Navarro-lisboa, R., & Dicastillo, C. L. De. (2016). Evaluation of polyphenol content and antioxidant capacity of fruits and vegetables using a modified enzymatic extraction. Food Technology and Biotechnology, 54(4), 462–467. https://doi.org/10.17113/ft b.54.04.16.4497.

Amarowicz, R. (2016). Natural phenolic compounds protect LDL against oxidation. *European Journal of Lipid Science and Technology, 118*(5), 677–679. https://doi.org/10.1002/ejlt.201600077

Ambrose-Oji, B.;. Urban Food Systems and African Indigenous Vegetables : Defining theSpaces and Places for African Indigenous Vegtables in Urban and Peri-Urban Agriculture. In African Indigenous Vegetables in Urban Agriculture; Shackleton, C., Pasquini, M., Drescher, A., Eds.; Routledge: London, 2009, pp 1–34. ISBN 9781136574993.

Amin, I., Norazaidah, Y., & Hainida, K. I. E. (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chemistry, 94*(1), 47–52. https://doi.org/10.1016/j.foodchem.2004.10.048

Bayliak, M. M., Burdyliuk, N. I., & Lushchak, V. I. (2016). Effects of pH on antioxidant and prooxidant properties of common medicinal herbs. *Open Life Sciences, 11*(1), 298–307. https://doi.org/10.1515/biol-2016-0040

Caicedo-Lopez, L. H., Luzardo-Ocampo, I., Cuellar-Nuñez, M. L., Campos-Vega, R., Mendoza, S., & Loarca-Piña, G. (2018). Effect of the in vitro gastrointestinal digestion on free-phenolic compounds and mono/oligosaccharides from Moringa oleifera leaves: Bioaccessibility, intestinal permeability and antioxidant capacity. Food Research International, (November). https://doi.org/10.1016/j.foodres.2018.11.017.

Caicedo-Lopez, L. H., Luzardo-Ocampo, I., Cuellar-Nuñez, M. L., Campos-Vega, R., Mendoza, S., & Loarca-Piña, G. (2019). Effect of the in vitro gastrointestinal digestion on free-phenolic compounds and mono/oligosaccharides from Moringa oleifera leaves: Bioaccessibility, intestinal permeability and antioxidant capacity. Food Research International, 120(July 2018), 631–642. https://doi.org/10.1016/j.foodres.2018.11.017.

Camilo López-Alarcón, C., & Denicola, A. (2013). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica Chimica Acta, 763*(February), 1–10. https://doi.org/10.1016/j.aca.2012.11.051

Campisi, A., Acquaviva, R., Raciti, G., Duro, A., Rizzo, M., & Santagati, N. A. (2019). Antioxidant activities of Solanum nigrum L. leaf extracts determined in in vitro cellular models. *Foods, 8*(63), 1–12. https://doi.org/10.3390/foods8020063

Chandrasekara, A., Daugelaite, J., & Shahidi, F. (2018). DNA scission and LDL cholesterol oxidation inhibition and antioxidant activities of Bael (*Aegle marmelos*) flower extracts. *Journal of Traditional and Complementary Medicine, 8*(3), 428–435. https://doi.org/10.1016/j.jtcme.2017.08.010

Chang, J. J., Chung, D. J., Lee, Y. J., Wen, B. H., Jao, H. Y., & Wang, C. J. (2017). *Solanum nigrum* polyphenol extracts inhibit Hepatic inflammation, oxidative stress, and lipogenesis in high-fat-diet-treated mice. *Journal of Agricultural and Food Chemistry, 65*(42), 9255–9265. https://doi.org/10.1021/acs.jafc.7b03578

Chen, Y., Ma, X., Fu, X., & Yan, R. (2017). Phytochemical content, cellular antioxidant activity and antiproliferative activity of *Adinandra nitida* tea (Shiyacha) infusion subjected to *in vitro* gastrointestinal digestion. *RSC Advances, 7*(80), 50430–50440. https://doi.org/10.1039/c7ra07429h

Cueva, C., Silva, M., Pinillos, I., Bartolomé, B., & Moreno-Arribas, M. V. (2020). Interplay between dietary polyphenols and oral and gut microbiota in the development of colorectal cancer. *Nutrients, 12*(3), 625. https://doi.org/10.3390/nu12030625

De Santiago, E., Pereira-Caro, G., Moreno-Rojas, J. M., Cid, C., & De Peña, M. P. (2018). Digestibility of (poly)phenols and antioxidant activity in raw and cooked cactus cladodes (*Opuntia ficus-indica*). *Journal of Agricultural and Food Chemistry, 66*(23), 5832–5844. https://doi.org/10.1021/acs.jafc.8b01167

Deiana, M., Serra, G., & Corona, G. (2018). Modulation of intestinal epithelium homeostasis by extra virgin olive oil phenolic compounds. *Food and Function, 9*(8), 4085–4099. https://doi.org/10.1039/c8fo00354h

Dianita, R., & Jantan, I. (2019). Inhibition of human platelet aggregation and low-density lipoprotein oxidation by *Premna foetida* extract and its major compounds. *Molecules, 24*(1469), 1–14. https://doi.org/10.3390/molecules24081469

Edith, A. A., Souleymane, M., Souleymane, T., & Honorine, K. A. (2018). Antioxidant potentialities of black nightshade and sweet potatoes leaves consumed in Côte d ' Ivoire. *American Journal of Food and Nutrition, 6*(1), 28–32. https://doi.org/10.12691/ajfn-6-1-5

Ghosh, S., Chakraborty, R., & Raychaudhuri, U. (2015). Determination of pH-dependent antioxidant activity of palm (*Borassus flabellifer*) polyphenol compounds by photoluminol and DPPH methods: A comparison of redox reaction sensitivity. *3. Biotech, 5*(5), 633–640. https://doi.org/10.1007/s13205-014-0260-7

Gonçalves, S., Moreira, E., Andrade, P. B., Valentão, P., & Romano, A. (2018). Effect of *in vitro* gastrointestinal digestion on the total phenolic contents and antioxidant activity of wild Mediterranean edible plant extracts. *European Food Research and Technology, 245*(2019), 753–762. https://doi.org/10.1007/s00217-018-3197-y

Gunathilake, K., Ranaweera, K., & Rupasinghe, H. (2018). Influence of Boiling, Steaming and Frying of Selected Leafy Vegetables on the In Vitro Anti-inflammation Associated Biological Activities. *Plants, 7*(1), 22. https://doi.org/10.3390/plants7010022

Gutiérrez-Grijalva, E. P., Antunes-Ricardo, M., Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., & Basilio Heredia, J. (2019). Cellular antioxidant activity and *in vitro* inhibition of α-glucosidase, α-amylase and pancreatic lipase of oregano polyphenols under simulated gastrointestinal digestion. *Food Research International, 116*(2018), 676–686. https://doi.org/10.1016/j.foodres.2018.08.096

Howard, L. R., Pandjaitan, N., Morelock, T., & Gil, M. I. (2002). Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *Journal of Agricultural and Food Chemistry, 50*(21), 5891–5896. https://doi.org/10.1021/jf020507o

Kapusta-Duch, J., Kusznierewicz, B., Leszczyńska, T., & Borczak, B. (2016). Effect of culinary treatment on changes in the contents of selected nutrients and non-nutrients in curly Kale (*Brassica oleracea* Var. acephala). *Journal of Food Processing and Preservation, 40*(6), 1280–1288. https://doi.org/10.1111/jfpp.12713

Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences, 11*(8), 982–991. https://doi.org/10.7150/ijbs.12096

Kayitesi, E. (2013). *Micronisation of cowpeas : The effects on sensory quality, phenolic compounds and bioactive properties*. University of Pretoria.

Kellett, M. E., Greenspan, P., & Pegg, R. B. (2018). Modification of the cellular antioxidant activity (CAA) assay to study phenolic antioxidants in a Caco-2 cell line. *Food Chemistry, 244*(2017), 359–363. https://doi.org/10.1016/j.foodchem.2017.10.035

Kumar, S., Krishna Chaitanya, R., & Preedy, V. R. (2018). Assessment of antioxidant potential of dietary components. In V. R. Preedy & R. R. Watson (Eds.), HIV/AIDS: Oxidative Stress and Dietary Antioxidants (1st ed., pp. 239–25). https://doi.org/10.1016/B978-0-12-809853-0.00020-1.

Lee, H. J., Cha, K. H., Kim, C. Y., Nho, C. W., & Pan, C. H. (2014). Bioavailability of hydroxycinnamic acids from *Crepidiastrum denticulatum* using simulated digestion and Caco-2 intestinal cells. *Journal of Agricultural and Food Chemistry, 62*(23), 5290–5295. https://doi.org/10.1021/jf500319h

Mavhungu, N. P. (2011). *Antioxidant properties and cellular protective effects of selected African green leafy vegetables*. University of Pretoria.

Mawalagedera, S. M. M. R., Ou, Z.-Q., Gould, K. S., McDowell, A., Gould, K. S., McDowell, A., & Gould, K. S. (2016). Effects of boiling and *in vitro* gastrointestinal digestion on the antioxidant activity of *Sonchus oleraceus* leaves. *Food Function, 7*(3), 1515–1522. https://doi.org/10.1039/C5FO01508A

Mncwango, N., Mavengahama, S., Ntuli, N., & Van Jaarsveld, C. (2020). Diversity, consumption dynamics and ethnomedical claims of traditional leafy vegetables consumed by a rural community in the KwaMbonambi area, northern KwaZulu-Natal, South Africa. *Biodiversitas Journal of Biological Diversity, 21*(3).

Moyo, S. M., Kayitesi, E., Mavumengwana, V., & Madala, N. E. (2016). Effects of cooking and drying on the total phenolic, total flavonoid content, antioxidant and antibacterial activity of Cleome gynandra (Spider plant). *International Conference on Advances in Science, Engineering, Technology & Natural Resources*, 136–141.

Moyo, S. M., Serem, J. C., Bester, M. J., Mavumengwana, V., & Kayitesi, E. (2020). Influence of boiling and subsequent phases of digestion on the phenolic content, bioaccessibility, and bioactivity of *Bidens pilosa* (Blackjack) leafy vegetable. *Food Chemistry, 311*. https://doi.org/10.1016/j.foodchem.2019.126023

Murador, D., Braga, A. R., Da Cunha, D., & De Rosso, V. (2018). Alterations in phenolic compound levels and antioxidant activity in response to cooking technique effects: A meta-analytic investigation. *Critical Reviews in Food Science and Nutrition, 58*(2), 169–177. https://doi.org/10.1080/10408398.2016.1140121

Murakami, M., Yamaguchi, T., Takamura, H., & Matoba, T. (2004). Effects of thermal treatment on radical-scavenging activity of single and mixed polyphenolic compounds. *Journal of Food Science, 69*(1), 1–5. https://doi.org/10.1111/j.1365-2621.2004.tb17848.x

Neugart, S., Baldermann, S., Ngwene, B., Wesonga, J., & Schreiner, M. (2017). Indigenous leafy vegetables of Eastern Africa - A source of extraordinary secondary plant metabolites. *Food Research International, 100*, 411–422. https://doi.org/10.1016/j.foodres.2017.02.014

Nwozo, S. O., Oso, B. J., & Oyinloye, B. E. (2015). Effect of heat on antioxidant activity of some tropical leafy vegetables. *Nigerian Journal of Basic and Applied Science, 23*(2), 93–101.

Palafox-Carlos, H., Ayala-Zavala, J. F., & González-Aguilar, G. A. (2011). The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *Journal of Food Science, 76*(1), 6–15. https://doi.org/10.1111/j.1750-3841.2010.01957.x

Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O. (2014). *In vitro* bioaccessibility of health-related compounds from a blended fruit juice-soymilk beverage: Influence of the food matrix. *Journal of Functional Foods, 7*(1), 161–169. https://doi.org/10.1016/j.jff.2014.01.023

Rosa, L. A. De, Moreno-escamilla, J. O., Rodrigo-garcía, J., & Alvarez-parrilla, E. (2019). Phenolic Compounds. In E. M. Yahia (Ed.), Postharvest Physiology and Biochemistry of Fruits and Vegetables (1st ed., pp. 253–272). https://doi.org/10.1016/B978-0-12-813278-4.00012-9.

Roy, M. K., Takenaka, M., Isobe, S., & Tsushida, T. (2007). Antioxidant potential, anti-proliferative activities, and phenolic content in water-soluble fractions of some commonly consumed vegetables: Effects of thermal treatment. *Food Chemistry, 103*(1), 106–114. https://doi.org/10.1016/j.foodchem.2006.08.002

Särkinen, T., Poczai, P., Barboza, G. E., Van Der Weerden, G. M., Baden, M., & Knapp, S. (2018). A revision of the old world black nightshades (Morelloid clade of *Solanum* L., Solanaceae). *PhytoKeys, 106*, 1–223. https://doi.org/10.3897/phytokeys.106.21991

Semwal, D. K., Semwal, R. B., Combrinck, S., & Viljoen, A. (2012). Myricetin: A dietary molecule with diverse biological activities. *Asian Journal of Chemistry, 24*(4), 1798–1804. https://doi.org/10.3390/nu8020090

Seraglio, S. K. T., Gonzaga, L. V., Schulz, M., Vitali, L., Micke, G. A., Costa, A. C. O., … Borges, G. D. S. C. (2018). Effects of gastrointestinal digestion models *in vitro* on phenolic compounds and antioxidant activity of juçara (*Euterpe edulis*). *International Journal of Food Science and Technology, 53*(8), 1824–1831. https://doi.org/10.1111/ijfs.13816

Serem, J. C. (2018). Identification and characterization of bioactivity of simulated gastrointestinal digested indigenous southern African honey samples. *University of Johannesburg*.

Serrano, J., Goñi, I., & Saura-Calixto, F. (2007). Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International, 40*(1), 15–21. https://doi.org/10.1016/j.foodres.2006.07.010

Shahidi, F., & Peng, H. (2019). Bioaccessibility and bioavailability of phenolic compounds. *Journal of Food Bioactives, 4*, 11–68. https://doi.org/10.31665/jfb.2018.4162

Shahidi, F., Vamadevan, V., Oh, W. Y., & Peng, H. (2019). Phenolic compounds in agri-food by-products, their bioavailability and health effects. *Journal of Food Bioactives, 5*, 57–119. https://doi.org/10.31665/JFB.2019.5178.

Shahidi, F., Zhong, Y., & Chandrasekara, A. (2012). Antioxidants and human health. In L. L. Yu, R. Tsao, & F. Shahidi (Eds.), Cereals and Pulses: Nutraceutical Properties and Health Benefits (1st ed., pp. 273–308). https://doi.org/10.1002/9781118229415.

Spínola, V., Llorent-Martínez, E. J., & Castilho, P. C. (2018). Antioxidant polyphenols of Madeira sorrel (*Rumex maderensis*): How do they survive to *in vitro* simulated gastrointestinal digestion? *Food Chemistry, 259*(March), 105–112. https://doi.org/10.1016/j.foodchem.2018.03.112

Subudhi, B. B., & Bhoi, A. (2014). Antioxidative effects of *Brassica juncea* and *Moringa oliefera* prepared by different processing methods. *Journal of Food Science and Technology, 51*(April), 790–794. https://doi.org/10.1007/s13197-011-0542-6

Tiwari, P., & Mishra, J. (2017). Role of flavonoids in DNA damage and carcinogenesis prevention. *Journal of Carcinogenesis & Mutagenesis, 8*(4), 4–9. https://doi.org/10.4172/2157-2518.1000297

Upadhyay, R., & Mohan Rao, L. J. (2013). An outlook on chlorogenic acids-occurrence, chemistry, technology, and biological activities. *Critical Reviews in Food Science and Nutrition, 53*(9), 968–984. https://doi.org/10.1080/10408398.2011.576319

Van Rensburg Willem, J., Voster, I.H., Van Zijl, J.J.B., & Sonja, L.V. (2007). Conservation of African leafy vegetables in South Africa. Water SA. 2007, 33(3).

Villa-Rodriguez, J. A., Ifie, I., Gonzalez-Aguilar, G. A., & Roopchand, D. E. (2019). The gastrointestinal tract as prime site for cardiometabolic protection by dietary polyphenols. *Advances in Nutrition, 1–13*. https://doi.org/10.1093/advances/nmz038

Wan, C., Li, S., Liu, L., Chen, C., & Fan, S. (2017). Caffeoylquinic acids from the aerial parts of *Chrysanthemum coronarium* L. *Plants, 6*(4), 10. https://doi.org/10.3390/plants6010010

Wang, J., Zhao, & Xi, H. (2016). Degradation kinetics of fisetin and quercetin in solutions affected by medium pH, temperature and co-existing proteins. *Journal of the Serbian Chemical Society, 81*(3), 243–253. https://doi.org/10.2298/JSC150706092W

Yang, I., Jayaprakasha, G. K., & Patil, B. (2018). *In vitro* digestion with bile acids enhances the bioaccessibility of kale polyphenols. *Food and Function, 9*(2), 1235–1244. https://doi.org/10.1039/c7fo01749a

Yang, M. Y., Hsu, L. S., Peng, C. H., Shi, Y. S., Wu, C. H., & Wang, C. J. (2010). Polyphenol-rich extracts from *Solanum nigrum* attenuated PKC α-mediated migration and invasion of hepatocellular carcinoma cells. *Journal of Agricultural and Food Chemistry, 58*(9), 5806–5814. https://doi.org/10.1021/jf100718b

Yujing, L., Zhaojie, L., Hu, H., Yongliang, Z., & Liping, S. (2018). Metal chelating, inhibitory DNA damage, and anti-inflammatory activities of phenolics from Rambutan (*Nephelium lappaceum*) peel and the quantifications of Geraniin and Corilagin. *Molecules, 23*(2263), 1–12. https://doi.org/10.3390/molecules23092263

Zhou, K., & Yu, L. (2006). Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. *LWT - Food Science and Technology, 39*(10), 1155–1162. https://doi.org/10.1016/j.lwt.2005.07.015