Changes in phenolic composition, antioxidant and antidiabetic properties of turmeric as affected by cooking time

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Abstract

This study sought to assess the effects of cooking time on the phenolic composition, antioxidant and antidiabetic properties of turmeric extracts. Steam cooked turmeric extracts were prepared by cooking 10 g of turmeric for 10 (SCT10) and 20 (SCT20) min, respectively, while the raw sample (RWT) was prepared by soaking 10 g of turmeric in 200 ml of distilled water for 1 h. The total phenol and flavonoid contents of the turmeric extracts were determined and phenolic composition was assessed using high performance liquid chromatography coupled with diode array detection (HPLC-DAD). The ferric reducing antioxidant property, radical [1,1-diphenyl-2 picrylhydrazyl (DPPH), nitric oxide (NO) and hydroxyl (OH)] scavenging abilities and interaction of the extracts with α-amylase and α-glucosidase activities were also investigated. SCT20 (4.26 mg/g and 3.96 mg/g) had significantly higher total phenol and flavonoid contents than SCT10 (3.58 mg GAE/g and 3.38 mg QE/g) and RWT (2.44 mg/g and 2.38 mg/g), respectively.

SCT20 (76.8 mmol/100 g) had the highest reducing property, while RWT (68.2 mmol AAE/100 g) had the lowest. Furthermore, SCT20 had significantly (p<0.05) higher DPPH, NO and OH radical scavenging ability than SCT10 and RWT. The extracts inhibited α-amylase and α-glucosidase in a dose-dependent manner. While RWT had the lowest inhibitory effects, SCT20 had the highest. The HPLC analysis revealed the presence of phenolic compounds such as gallic acid, caffeic acid, catechin, quercetin, rutin, luteolin and curcumin. The phenolic constituents of the steam cooked extracts were significantly higher than those of the raw samples except for caffeic acid which decreased with increased cooking time. Therefore, steam cooking increased the phenolic composition and antioxidant properties as well as the α-amylase and α-glucosidase inhibitory activities of turmeric.

Introduction

Turmeric is a commonly used spice obtained from Curcuma longa L., a rhizomatous herbaceous plant of the ginger family, which is widely cultivated in tropical and subtropical regions of the world [1]. In Ayurvedic and traditional Chinese medicine, turmeric is used for the treatment of inflammatory conditions and as a stimulant, carminative, astringent and diuretic [2-4]. Some reports have revealed that turmeric extracts have cardio-protec-
tive, hypoglycaemic, anti-amyloidogenic, antimicrobial, anticancer and antioxidant activity [5-10]. Turmeric is known to be a good source of natural antioxidants that can protect cells from oxidative damage [11,12]. Oxidative stress, which is characterized by free radical generation, has been linked to the aetiology and pathophysiology of type 2 diabetes. High blood glucose is a major feature of type 2 diabetes and control of post-prandial blood glucose requires inhibition of carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase), which is a good therapeutic approach for the management and/or treatment of type 2 diabetes. Phytochemical compounds with strong antioxidant properties are known to be strong inhibitors of these enzymes and it has been demonstrated that turmeric has promising potential [13,14]. Turmeric is commonly consumed in raw or cooked form. Various reports have revealed that heat processing of some spices and vegetables could lead to loss of some bioactive compounds, which can reduce their functional and medicinal properties [15-17]. However, conflicting reports have revealed that some heat processing methods can increase the phenolic content and antioxidant activity of spices, fruits and vegetables [18,19]. Nevertheless, to the best of our knowledge there is little or no information about the effect of cooking on the antioxidant and antidiabetic properties of turmeric. This study therefore sought to investigate the effect of steam cooking on the phenolic composition, antioxidant and antidiabetic properties of turmeric extracts.

Materials and methods

Extract collection and identification
Dried turmeric (Curcuma longa L.) rhizomes were purchased at Oja-Oba market in Akure city, Nigeria. Authentication of the sample was carried out in the Department of Biology, Federal University of Technology, Akure, Ondo State, with voucher number FUTA/BIO/0139.

Extract preparation
Turmeric (10 g) was steam cooked for 10 min (SCT10) and 20 min (SCT20), respectively, while 10 g of raw turmeric (RWT) was soaked in 200 ml of distilled water for 1 h. The samples were allowed to cool and filtered with filter paper. The filtrates from RWT, SCT10 and SCT20 were kept in a refrigerator until analysis.

Chemicals and reagents
Thiobarbituric acid (TBA), 1,10-phenanthroline, deoxyribose, gallic acid and Folin–Ciocalteau’s reagent were procured from Sigma-Aldrich (St Louis, MO), while trichloroacetic acid (TCA) was from Sigma-Aldrich Chemie (Steinheim, Germany). Hydrogen peroxide, methanol, sodium nitroprusside, Griess reagent, acetic acid, hydrochloric acid, sodium carbonate, aluminum chloride, potassium acetate, sodium dodecyl sulphate, iron (II) sulfate, potassium ferricyanide and ferric chloride were from BDH Chemicals (Poole, UK). Unless stated otherwise, all the chemicals and reagents used were of analytical grades and the water was glass distilled.

Determination of total phenol content
Total phenol content was determined according to the method of Singleton et al [20]. Briefly, 0.5 ml extracts were oxidized with 2.5 ml 10 % Folin-Ciocalteau’s reagent (v/v) and neutralized with 2.0 ml 7.5 % sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in a spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content
The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda et al [21]. Briefly, 0.5 ml appropriately diluted extracts (1:10) were mixed with 2.5 ml 10 % Folin-Ciocalteau’s reagent (v/v) and neutralized with 2.0 ml 7.5 % sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in a spectrophotometer. The total flavonoid content was subsequently calculated as quercetin equivalent.

Quantification of compounds by high performance liquid chromatography coupled with diode array detection (HPLC-DAD)
Reverse phase chromatographic analyses were car-
ried out under gradient conditions using a C_{18} column (4.6 mm x 150 mm) packed with 5 μm diameter particles; the mobile phase was water containing 2 % acetic acid (A) and methanol (B), and the composition gradient was: 5% B until 2 min and 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 80 min, respectively, following the method described by Adefegha et al [22] with slight modifications. RWT, STC10 and SCT20 extracts were analyzed at a concentration of 15 mg/ml. The presence of nine antioxidant compounds was investigated, namely, gallic acid, caffeic acid, catechin, epicatechin, quercetin, quercitrin, rutin, luteolin and curcumin. These compounds were identified by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.6 ml/min, injection volume 50 μl and the wavelengths were 270 nm for gallic acid, 281 nm for catechin and epicatechin, 327 nm for caffeic acid, 365 nm for quercetin, quercitrin, rutin and luteolin, and 426 nm for curcumin. The extracts and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed in an ultrasonic bath before use. Stock solutions of reference standards were prepared in the HPLC mobile phase at a concentration range of 0.030–0.200 mg/ml for catechin, epicatechin, quercetin, quercitrin, rutin, curcumin and luteolin, and 0.025–0.300 mg/ml for caffeic and gallic acids. The chromatography peaks were confirmed by comparing their retention times with those of reference standards and by DAD spectra (200–500 nm). Calibration curves were as follows: for catechin: Y = 11965x + 1273.8 (r = 0.9999); epicatechin: Y = 13065x + 1187.6 (r = 0.9996); gallic acid: Y = 11890x + 1193.7 (r = 0.9997); caffeic acid: Y = 12549x + 1307.2 (r = 0.9998); rutin: Y = 12853x + 1238.9 (r = 0.9991); quercetin: Y = 13574x + 1271.9 (r = 0.9995); quercitrin: Y = 11965x + 1358.7 (r = 0.9995); curcumin: Y = 12895x + 1289.8 (r = 0.9999); and luteolin Y = 13509x + 1185.4 (r = 0.9998). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves; LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

**Determination of ferric reducing antioxidant property**

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce FeCl₃ solution as described by Oyaizu [23]. An aliquot (500 μl) of the extracts was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 801 × g for 10 min. A 5 ml aliquot of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm and the ferric reducing power was subsequently calculated using ascorbic acid equivalent.

**Determination of 1, 1-diphenyl-2-picrylhydrazyl radical scavenging ability**

The free radical scavenging ability of the extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was evaluated as described by Gyamfi et al [24]. Briefly, an appropriate dilution of the extracts (1 ml) was mixed with 1 ml 0.4 mM DPPH radicals in methanolic solution. The mixture was left in the dark for 30 min, and the absorbance was taken at 516 nm. The control was carried out by using 2 ml DPPH solution without the test extracts. The DPPH radical scavenging ability was subsequently calculated as a percentage of the control:

\[
\text{DPPH radical scavenging ability (%) = } \frac{(\text{Abs ref} - \text{Abs sam})}{\text{Abs ref}} \times 100
\]

where Abs ref represents the absorbance of the reference and Abs sam represents the absorbance of the sample.

**Nitric oxide radical scavenging ability**

The scavenging effect of the extracts on nitric oxide (NO·) radical was measured according to the method of Susanta et al [25]. Turmeric extract (100–400 μl) was added to the test tubes containing 1 ml of sodium nitroprusside solution (25 mM) and tubes were incubated at 37°C for 2 h. An aliquot (0.5 ml) of the incubation was removed.
and diluted with 0.3 ml Griess reagent (1% sulphanilamide in 5% H₃PO₄ and 0.1% naphthyleylene diaminedihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank:

\[
\text{NO radical scavenging ability (\%)} = \left[\frac{(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})}{\text{Abs}_{\text{ref}}}\right] \times 100
\]

where \(\text{Abs}_{\text{ref}}\) represents the absorbance of the reference, and \(\text{Abs}_{\text{sam}}\) represents the absorbance of the sample.

\(\text{OH radical scavenging ability}\)

The ability of the extracts to prevent Fe\(^{2+}/\text{H}_2\text{O}_2\)-induced decomposition of deoxyribose was evaluated using the method of Halliwell and Gutteridge [26]. Briefly, appropriate dilutions of the extracts were added to a reaction mixture containing 120 μl 20 mM deoxyribose, 400 μl 0.1 M phosphate buffer, 40 μl 20 mM hydrogen peroxide and 40 μl 500 μM FeSO₄, and the volume made up to 800 μl with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by adding 0.5 ml 2.8% trichloroacetic acid (TCA). After this, 0.4 ml 0.6% thiobarbituric acid (TBA) solution was added to the test tubes which were then incubated in a water bath for 20 min. The absorbance was measured at 532 nm using a spectrophotometer. The percentage (%) hydroxyl radical scavenging ability was subsequently calculated:

\[
\text{OH radical scavenging ability (\%)} = \left[\frac{(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})}{\text{Abs}_{\text{ref}}}\right] \times 100
\]

where \(\text{Abs}_{\text{ref}}\) represents the absorbance of the reference and \(\text{Abs}_{\text{sam}}\) represents the absorbance of the sample.

\(\alpha\)-Amylase inhibition assay

\(\alpha\)-Amylase inhibition was determined by the method of Bernfield [27]. The extracts (0–200 μl) and 500 μl of 0.02 M sodium phosphate buffer (pH 6.9) containing porcine pancreatic \(\alpha\)-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25°C for 10 min. Then, 500 μl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixture was incubated at 25°C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid colour reagent.

Thereafter, the mixture was incubated in a water bath at 100°C for 5 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm. The \(\alpha\)-amylase inhibitory activity was expressed as percentage inhibition:

\[
\% \text{Inhibition} = \left[\frac{(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})}{\text{Abs}_{\text{ref}}}\right] \times 100
\]

where \(\text{Abs}_{\text{ref}}\) is the absorbance without the extracts and \(\text{Abs}_{\text{sam}}\) is the absorbance of the extracts.

\(\alpha\)-Glucosidase inhibition assay

The extracts (0–200 μl) and 100 μl \(\alpha\)-glucosidase (EC 3.2.1.20) solution in 0.1 M phosphate buffer (pH 6.9) was incubated at 25°C for 10 min. Then, 50 μl 5 mM p-nitrophenyl-\(\alpha\)-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25°C for 5 min, before absorbance was read at 405 nm in the spectrophotometer. The \(\alpha\)-glucosidase inhibitory activity was expressed as percentage inhibition [28]:

\[
\% \text{Inhibition} = \left[\frac{(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}})}{\text{Abs}_{\text{ref}}}\right] \times 100
\]

where \(\text{Abs}_{\text{ref}}\) represents the absorbance without the extracts and \(\text{Abs}_{\text{sam}}\) represents the absorbance of the extracts.

Data Analysis

The results of three replicates were pooled and expressed as the mean ± standard deviation (SD). Student’s \(t\) test, one-way analysis of variance (ANOVA) and least significance difference (LSD) were performed. Significance was accepted at \(p\leq0.05\). EC\(_{50}\) (effective concentration causing 50% inhibition) was determined using linear regression analysis.

Results and Discussion

The total phenol and flavonoid contents of the raw and cooked turmeric extracts were reported as gallic acid equivalents (GAE) and quercetin equivalents (QE), respectively, and are presented in Figs. 1 and 2. The results show that SCT20 (4.26 mg GAE/g and 3.96 mg QE/g) had significantly (\(p<0.05\)) higher total phenolic and flavonoid contents than SCT10 (3.58 mg GAE/g and 3.38 mg QE/g) and RWT (2.44 mg GAE/g and 2.38 mg QE/g).
This finding indicates that steam cooking increased the phenolic content of the spice. The result correlates with previous reports where increased cooking time caused an increase in the phenolic content of vegetables [29]. However, there are contradictory reports that thermal processing decreases the phenolic content of fruits and vegetables [30,31], although Hunter and Fletcher [32] reported that the effect of thermal processing on phenolics depends on the type of plant food. The increase in the phenolic content of the cooked turmeric extracts could be attributed to increased levels of free phenolics and flavonoids released by the heat treatment.

The ferric reducing antioxidant properties of the turmeric extracts, reported as ascorbic acid equivalent (AAE) as shown in Fig. 3, revealed that SCT$_{20}$ (76.8 mmol AAE/100 g) had significantly (p<0.05) higher reducing power than SCT$_{10}$ (72.4 mmol AAE/100 g) and RWT (68.2 mmol AAE/100 g). This result revealed that as steam cooking time increases, the reducing property of the turmeric extracts increases. The result also agrees with the trend observed in the total phenol and flavonoid contents of the extracts and previous reports where heat processing increased the reducing capacity of vegetables via the reduction of Fe$^{3+}$ to Fe$^{2+}$ [29].

The DPPH radical scavenging abilities of the RWT, SCT$_{10}$ and SCT$_{20}$ extracts are shown in Fig. 4. The extracts scavenged DPPH in a dose-dependent manner; however, SCT$_{20}$ (EC$_{50}$=2.72 mg/ml) had significantly higher (p<0.05) scavenging ability than SCT$_{10}$ (EC$_{50}$=3.12 mg/ml) and RWT (EC$_{50}$=3.83 mg/ml) as shown in Table 1. Furthermore, the extracts scavenged NO radicals in a dose-dependent manner as shown in Fig. 5.
The result revealed that the heat treatment increased radical scavenging ability as higher scavenging abilities were observed in \( \text{SCT}_{20} \) (EC_{50}=0.13 mg/ml) and \( \text{SCT}_{10} \) (EC_{50}=0.47 mg/ml) compared to RWT (EC_{50}=0.55 mg/ml). The results in Fig. 6 and Table 1 also revealed that the turmeric extracts scavenged OH radicals in a dose-dependent manner. \( \text{SCT}_{20} \) (EC_{50}=0.13 mg/ml) had a higher scavenging ability compared to \( \text{SCT}_{10} \) (EC_{50}=0.15 mg/ml) and RWT (EC_{50}=0.18 mg/ml).

Free radical generation has been associated with hyperglycaemia-induced diabetic complications. Hyperglycaemia stimulates the formation of ROS/reactive nitrogen species (RNS) such as NO and OH radicals. NO radicals can react with superoxide to form peroxynitrite, a powerful oxidant which has been implicated in diabetes [33]. Also, glucose can react with hydrogen peroxide in the presence of iron to form hydroxyl radical which can attack and reduce pancreatic \( \beta \)-cell function [34]. The increased DPPH, NO and OH radical scavenging abilities observed in the SCT extracts can be linked to the increase in the phenolic and flavonoid content as the antioxidative activity of polyphenolic compounds has been attributed to radical scavenging ability [35].

Inhibition of carbohydrate hydrolyzing enzymes is a good therapeutic approach in the management of diabetic conditions. The effect of the raw and cooked extracts of turmeric on \( \alpha \)-amylase activity is presented in Fig. 7. The extracts inhibited \( \alpha \)-amylase activity in a dose-dependent manner; however, \( \text{STC}_{20} \) (EC_{50}=0.85 mg/ml) had a significantly higher (p<0.05) inhibitory effect on \( \alpha \)-amylase activity than \( \text{SCT}_{10} \) (EC_{50}=1.01 mg/ml)
However, SCT10 had the highest amount of caffeic acid (5.36 mg/g), while there was no significant (p>0.05) difference in the gallic acid content of the three extracts. This result shows that cooking increased the phenolic constituents of the extracts, although a decrease was observed for gallic acid and caffeic acid in SCT20. This finding shows the same ml) and RWT extract (EC50=1.13 mg/ml) (Table 1). The effect of the turmeric extracts on α-glucosidase activity is presented in Fig. 8 and Table 1. RWT (EC50=1.16 mg/ml) extract had a significantly (p<0.05) lower α-glucosidase inhibitory activity compared to SCT10 (EC50=0.99 mg/ml) and SCT20 (EC50=0.86 mg/ml). The inhibitory effects of the extracts could be beneficial in the management and/or treatment of type 2 diabetes as they delay glucose absorption and reduce post-prandial hyperglycaemia. The result shows that cooking time increased the inhibitory activity of the extracts. This result agrees with the findings of Oboh et al [36] who reported that heat treatment such as roasting and cooking increased the α-amylase and α-glucosidase activities of African pear (Dacryodes edulis). The enzyme inhibitory activity of the extracts can be linked to their phenolic constituents such as caffeic acid, curcumin, luteolin, quercetin, rutin, quercitrin and epicatechin which were identified in the turmeric extracts and have been established by previous findings to inhibit α-amylase and α-glucosidase activity [37-40]. The phenolic composition of the turmeric extracts is also presented in Table 2. HPLC analysis (Fig. 9) revealed the presence of phenolic compounds such as curcumin, gallic acid, caffeic acid, catechin and epicatechin, and flavonoids such as rutin, quercetin, quercitrin and luteolin. The trend observed in these results shows that cooking increased the phenolic constituents of the extracts. SCT20 had a significantly (p<0.05) higher amount of curcumin (14.03 mg/g), catechin (5.93 mg/g), epicatechin (1.62 mg/g), rutin (2.09 mg/g), quercetin (11.73 mg/g), quercitrin (6.12 mg/g) and luteolin (10.36 mg/g) than SCT10 and RWT. However, SCT10 had the highest amount of caffeic acid (5.36 mg/g), while there was no significant (p>0.05) difference in the gallic acid content of the three extracts. This result shows that cooking increased the phenolic constituents of the extracts, although a decrease was observed for gallic acid and caffeic acid in SCT20. This finding shows the same
in temperature associated with the cooking time which can lead to the degradation of these phenolic acids. Lemos et al [41] reported that an increase in temperature also decreased gallic acid and caffeic acid content in baru nuts.

Conclusion

Our findings revealed that steam cooking increased the phenolic constituents, antioxidant and antidiabetic properties of turmeric. As cooking time increased, the phenolic composition, radical scavenging abilities and enzyme inhibitory activities of STC_{10} and STC_{20} increased. Therefore, steam cooked turmeric could be more beneficial in the management and/or treatment of diabetes than raw turmeric.

Conflict of interest
Authors declare no conflict of interest

trend for the total phenol and flavonoid contents of the turmeric extracts. The decrease observed in the gallic acid and caffeic acid contents in the SCT_{20} extract could be attributed to the increase
References