EFFECT OF COOKING METHODS ON POLYPHENOL, ANTIOXIDANT AND INHIBITION OF KEY ENZYMES LINKED TO CARBOHYDRATE METABOLISM OF COCOYAM (*COLOCASIA ESCULENTA* L. SCHOTT)

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Abstract
Roasting and boiling are two of the domestic cooking methods which affect the phytochemical, nutritional and the overall health functionality of food. The present study evaluated the effect of different cooking methods on the polyphenol, antioxidant and inhibition of key enzymes linked to carbohydrate metabolism of Cocoyam. Polyphenol contents were determined using total phenolics and total flavonoid, antioxidant capacity using total antioxidant capacity, ascorbic acid, ferric reducing power, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric-induced lipid peroxidation inhibition while ability to inhibit α-amylase and α-glucosidase enzymes were assayed to determine it effect on carbohydrate metabolism. The results showed that total phenolic content, total flavonoid content, total antioxidant capacity, DPPH and reducing power were significantly ($P < 0.05$) increased in roasted sample while ascorbic acid content was significantly decreased in both treatments. Also the roasted sample exhibited highest inhibition of α-glucosidase, α-amylase and induced lipid peroxidation which is significantly ($P < 0.05$) different from raw and boiled samples. Pearson correlation test exhibited positive correlation among polyphenols content, antioxidant activities and enzymes inhibition. These results showed that roasting enhanced the polyphenol, antioxidant and inhibition of enzymes linked to diabetes mellitus and therefore, it could be used by food industries to enhance the polyphenolic and antioxidant contents of foods.

Keywords: Roasting; Boiling; health functionality; Polyphenol; Cocoyam

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1. INTRODUCTION

Tuber is an important part of traditional diets and excellent source of carbohydrate throughout the tropics. Apart from its nutritional importance, it's also serves as a reservoir of vast phytochemicals with numerous biological activities such as antioxidant, antidiabetic and antimicrobial. One of the important tuber crops in tropics is Cocoyam (*Colocasia esculenta* L. Schott). It is a root crop belonging to the family of the Araceae, after yam and cassava, cocoyam is the third most important tuber crop in West Africa (Nwanekezi et al. 2010). Despite its adaptation to tropical conditions, it has long been cultivated in some part of Europe and Mediterranean (Oscarsson et al., 2007). Presently in subtropical and tropical regions, it is being cultivated for its edible carbohydrate-rich tuber, which is the most eaten part of the plant and are formed underground (Simsek and Nehir El 2015). Like other tuber crops, the unprocessed corm has low amount of fat (0.2%) and protein (1.5%) (Amon et al., 2011). It is a good source of fiber (0.8%), starch (70-80%) and ash (1.2%) (Jane et al., 1992). Starch obtained from cocoyam tuber is unique because of its small granular sizes ranging from 1 to 5 µl. The unique small granules coupled with high soluble dietary fiber content and low glycemic index makes cocoyam tuber a good source of carbohydrate for extruded special products such as low glycemic index foods and infant weaning diets (Huang et al., 2000). The fleshy tuber can be boiled, roasted or mashed into a meal and used as staple food or snack. Among the root tubers, cocoyam is widely prepared or processed into more consumable products such as chips, cereal bars, canned

products, paste, beverage powders, and flour (FAO, 2008; Simsek and Nehir El 2015). Cocoyam is usually subjected to a suitable method of processing in order to improve its nutritional value. Examples of such methods of processing are cooking and roasting. Proper cooking eliminates the harsh and sharp irritation of cocoyam tuber in the throat and mouth (Iwuoha and Kalu 1995) while roasting has been reported to improve the digestibility, colour, flavour, shelf life and reduces the antinutritional factors of foods (Gahalawat and Sehagal, 1992). However, most of these common methods of processing have been reported to alter polyphenolic contents, antioxidant capacities and health promoting properties of various foods, fruits and vegetables (Vadivel et al., 2015; Patras et al. 2009). The alteration in the total antioxidant capacity due to different methods of processing is of prime importance especially its impact on health functionality, palatability and consumer's acceptability.

Carbohydrate metabolism has been linked to the onset of diabetes mellitus (DM) which is a metabolic disease. It is characterized by excessive increase in blood sugar (hyperglycemia). The excessive increase in blood glucose is an outcome of both insulin insufficiency and insulin resistance. Presently it has become a global menace affecting about 425 million people globally (IDF 2017). Abnormal high glucose concentration in the blood usually causes excessive generation of free radicals which culminated into oxidative stress in the body (Chan et al., 2009; Wu et al., 2004). This oxidative stress is a major player in complications associated with diabetes mellitus. Apart from insulin resistance and insulin insufficiency, one factor that contributes to postprandial blood glucose concentration is the excessive activity of two pancreatic enzymes; amylase and glucosidase. The inhibition of these two enzymes has been considered a therapeutic target for the treatment of diabetes mellitus (Kim et al., 2011). Therefore any food that can inhibit these enzymes could be used to control blood glucose level in diabetic patients.

The aim of the present study was to evaluate the possible effect of domestic cooking methods (boiling and roasting) on antioxidant, antidiabetic and antiperoxidative properties of Cocoyam (*Colocasia esculenta* L. Schott).

2. MATERIALS AND METHODS

Sample collection

Fresh tubers of Cocoyam used in this work were bought from a traditional market, Okitipupa. The tubers were identified and authenticated at the Department of Biological Sciences Herbarium, Ondo State University of Science and Technology, Okitipupa (OSUSTECH). They were at full commercial maturity and eating qualities. They were fresh and without infection.

Cooking Treatments

The samples were prepared by the method earlier described by (Eleazu et al., 2016) with minor modifications. The tubers were washed and air-dried (1hr) and then divided into three portions (250g each). One portion was left raw (uncooked), stored at room temperature until use within 24 hr.

Boiling treatment

Briefly, 250 g of cocoyam was added to a boiling in water (500 ml) in a covered stainless steel pot and cooked for 20 mins on electric stove. After cooking, the sample was drained off and allowed cooling at room temperature for 2 hr. The cooled samples were oven dried at 40^{o}C for 18hrs. After 18 hrs, they were milled to particle size using laboratory blender. The milled samples were then sieved by 0.2mm sieve to obtain flour of uniform diameter.

Roasting treatment

The third portion (250 g) was processed by roasting on metal gauze for 20 mins on charcoal until slightly brown, during roasting the samples were turned around. After roasting, the sample was allowed cooling at room temperature for 2 hr. The cooled samples were oven dried at 40^{o}C for 18hrs. After 18 hrs, they were milled to particle size using laboratory blender. The milled samples were then sieved

by 0.2 mm sieve to obtain flour of uniform diameter.

Extraction of the sample

Ten grams (10 g) of powdered cocoyam sample was extracted with 100 ml distilled water at room temperature on orbital shaker (300 rpm for 1 hr). The resulting slurry was centrifuged at 866×g for 10 mins. The supernatants obtained were decanted and used for chemical analyses immediately.

Biochemical assays

Total phenolic content (TPC) determination

TPC of the thermally processed samples was determined using the modified Folin-Ciocalteu phenol reagent method (Kim et al., 2003). Briefly, 1 ml of the sample was mixed with 1 ml (10 %) of Folin-Ciocalteu phenol reagent. After 5 mins, 5 ml of 7 % sodium carbonate was added, immediately followed with 5ml of distilled water and shaken thoroughly. The mixture was left in the dark at room temperature for 90 minutes. The absorbance was measured at 750 nm and the TPC was evaluated from gallic acid standard curve.

Total flavonoid (TFC) determination

TFC of the thermally processed sample was determined using the method of (Park et al., 2008). Briefly, 0.3 ml (1mg/ml) of the sample was mixed with 3.4 ml of (30 %) methanol, 0.15 ml of (0.5 M) sodium nitrite and 0.15 ml of (0.3 M) aluminium chloride consecutively. After 5 mins, 1 ml of 1M sodium hydroxide was added to the reaction mixture and shaken together. The absorbance was measured at 506 nm and the flavonoid content was evaluated from quercetin standard curve

Total antioxidant capacity (TAC) determination

TAC of thermally processed sample was determined using the Phosphomolybdate method of (Prieto et al., 1999). Briefly, 0.1 ml (mg/ml) of sample is combined with 1ml of reagent (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid). The samples were incubated in a boiling water bath for 90 minutes at 95^{o}C. The samples were allowed to cool at room temperature, before the absorbance was measured at 695 nm and the total antioxidant capacity was calculated from ascorbic acid standard curve.

1,1-diphenyl-2-picrylhydrazyl scavenging activity (DPPH) determination

DPPH radical scavenging activity of thermally processed sample was determined using the method of (Gyamfi et al., 1999) using minor modification. Briefly, 4 ml of (30mg/l) DPPH solution prepared in methanol was added to 1 ml (1mg/ml) of sample. The samples were thoroughly mixed and allowed to stand for 30 minutes in the dark. The absorbance was measured at 520nm. The inhibition percentage was calculated as:

Inhibition percentage of DPPH = {(Abs control- Abs Sample)/ (Abs Control)} × 100

DPPH solution was used as control.

Ferric Reducing power (RP) determination

FRP of thermally processed sample was determined using the method of (Oyiazu 1986). Briefly, 1 ml (1mg/ml) phosphate buffer (0.2M, pH 6.6) was added to1 ml of the sample followed immediately by 1 ml potassium ferricyanide (1 %) and the reaction mixture was incubated for 20 minutes at 50^{o}C. The termination of the reaction was done by addition of 1ml (10 %) trichloroacetic acid. Briefly 1 ml upper portion of the sample was taken, added to 1 ml distilled water and immediately followed by (0.1%) 1.0 ml of ferric chloride. The reaction mixture was shaken together and absorbance was read at 700 nm and the ferric reducing power was evaluated from Ascorbic acid standard curve.

Ascorbic acid (AsA) determination

AsA of the thermally processed sample was determined using phosphotungstin method of (Rutkowski et al., 2007). Briefly, 2 ml of phosphotungstin reagent was added to 1 ml of the sample. The reaction mixture was allowed for 10 minutes before it was centrifuged for 10 minutes at 866×g. The absorbance of the supernatant was read at 700 nm and the ascorbic content of the sample was calculated.

Ascorbic content = (Abs of the Sample /Abs of Standard) × concentration of Standard

Amylase inhibition (AI) assay

AI of the thermally processed sample was

determined using the method (Worthington 1993). Briefly, 500 µl of sample and 500 µl (0.02 M) of phosphate buffer (0.006 M NaCl, pH 6.9) and containing 0.5 mg/ml α-amylase solution were added together and allowed to stand for 10 min at room temperature. Thereafter, 500 µl (1%) of starch solution prepared with 0.02 M phosphate buffer (0.006 M sodium chloride, pH 6.9) was added. The reaction mixtures were then incubated for 10 minutes at 25^0 C. The reaction was stopped with 1.0 ml (96 mM) of dinitrosalicylic acid. The reaction mixtures were then incubated in a boiling water bath for 5 min and allowed to cool at room temperature. The absorbance was read at 540 nm and percentage inhibition was calculated:

Inhibition percentage = {(Abs Control- Abs Sample)/ (Abs Control)} × 100

Glucosidase inhibition (GI) assay

GI of the thermally processed sample was done using the method of (Apostolidis et al., 2007). Briefly, 500µl of the sample and 1000 µl α-glucosidase (1.0 U/L) solution prepared with 0.1 M sodium phosphate buffer (pH 6.9) were added and pre-incubated for 10 minute at 25°C. Thereafter, 500µl (5 mM) nitrophenyl-glucopyranoside solution prepared with 0.1M sodium phosphate buffer (pH 6.9) was added and left to stand at room temperature for 5 minute. The absorbance of the reaction mixture was read at 405 nm and percentage inhibition was calculated:

Inhibition percentage = {(Abs Control - Abs Sample) / (Abs Control)} × 100

Lipid peroxidation (LP) inhibition

Preparation of Pancreas homogenate: The preparation of pancreas homogenate was done by the modified method of (Akinyemi et al., 2013). The rats were euthanized with pentobarbitone anesthesia and the pancreas was excised and weighed on ice. The pancreas tissue was immediately homogenized on ice-cold normal saline. The tissue homogenate was centrifuged at 866×g for 10 min. The lipid-rich supernatant obtained was used for the assay. The experimental procedures were conducted in line with procedure approved by University of Ibadan Animal Care Use and Research Ethics Committee for care and use of experimental animals.

Lipid peroxidation assay: LP of thermally processed samples was determined using the modified method of (Ohkawa et al., 1973). Briefly 200µl of the tissue homogenate was added to 30µl of 0.1M Tris-HCl buffer (pH 7.4), 100µl appropriate dilutions of sample and 30µl of the freshly prepared pro-oxidant solution (25µM ferrous sulphate). The reaction mixture was incubated for 2h at 37ºC. Thereafter 300µl sodium dodecyl sulphate (8.1%), 600µl acetic acid (pH 3.4) and 600µl thiobarbituric acid (0.8%) were added. The reaction mixture was incubated at 100^0C for 1h. The absorbance of malondialdehyde formed was read at 532 nm and percentage inhibition of lipid peroxidation was calculated by the method (Banerjee et al 2015).

Statistical analysis

Data are expressed as the mean ± SD of triplicate measurements. The significance between means of the samples were established by the analysis of variance using least significant difference (LSD) $P< 0.05$, charts were drawn with GraphPad prism 5 and Pearson correlation test was conducted to determine the correlation among polyphenols content, antioxidant activities and enzymes inhibition. Significant levels were established using $P< 0.05$.

3. RESULTS AND DISCUSSION

The effect of cooking methods (boiling and roasting) on total phenolics, total flavonoid, total antioxidant capacity and ascorbic acid was presented in Table 1. There was significant difference in the TPC. Total phenolic content was significantly decreased in the boiled sample but was increased in roasted sample when compared with raw sample. The same trend was also observed for TFC where roasted sample possessed significant higher content. In the TAC, there was a significant reduction in the thermally processed samples though the roasted sample exhibited significantly higher content than the cooked sample.

Table 1: Effect of thermal processing on TPC, TFC, TAC and AsA of *Colocasia esculentum*

	TPC (mg GAE/100g)	TFC (mg QUE/100g)	TAC (mg AsAE/100g)	AsA (mg/g)
RC	42.65.±0.32[a]	24.80±0.24[a]	52.19±0.40[a]	112.30±1.24[a]
BC	39.60±0.11[b]	18.10±0.01[b]	38.09±0.25[c]	27.19±0.15[b]
RSC	45.65±0.50[a]	26.45±0.39[a]	44.33±0.33[b]	28.94±0.09[b]

Values represent Mean± SD. Values with different letter across the column are significantly (P < 0.05) different. RC: Raw Cocoyam, BC: Boiled Cocoyam and RSC: Roasted Cocoyam. TPC: total phenolic content, TFC: total flavonoid content, TAC: total antioxidant capacity and AsA: ascorbic acid.

Many studies have reported conflicting results on the effects of heat treatment on foods. Some researchers have reported reduction in nutritional quality such as loss of polyphenolic compounds, antioxidative abilities and health functionality of heat-treated foods (Randhir et al., 2008) others have reported enhancement of polyphenolic compounds, antioxidative abilities and health functionality upon heat treatment (Dolinsky et al., 2016; Karigidi et al., 2018).

The decrease in polyphenol content after domestic cooking of cocoyam might be due to thermally induced degradation of phenolic compounds (Randhir et al., 2008) or leaching of water soluble polyphenol into the cooking water (Mazzeo et al., 2011). The data obtained in this work is in agreement with the study of (Mazzeo et al., 2011; Pellegrini et al., 2010), where boiling caused significant reduction in total phenolic and flavonoids contents of carrots and broccoli respectively. Whereas the polyphenol content of roasted sample increased, the increase might be due to non contact with water unlike boiling which prevents leaching of water soluble polyphenol hence the increase in polyphenol content (Mazzeo et al., 2011). Also the enhanced polyphenolic content could be due to formation of other phenolics apart from the endogenous ones as by-products during roasting treatment in the cocoyam (Randhir et al., 2008) or mobilization of bound polyphenolic from the breakdown of cellular components and cell walls (Karigidi et al., 2018). The browning pigments formed in roasted sample might also contribute to the increase in polyphenolic content and antioxidant abilities (Benjakul et al., 2005). This present study agreed with the work of Chandrasekara and Shahidi (2011) which reported increased polyphenol content in cashew nuts, kernels and testa upon roasting. The result of ascorbic acid evaluation showed that thermal processing decreases the content of ascorbic acid in the samples. The raw sample has significantly higher ascorbic acid content when compared with the processed samples. The reduction in the content of this bioactive compound could be due to its high sensitivity to heat and oxidation during processes of boiling and roasting (Mazzeo et al., 2011; Benjakul et al., 2005). Our finding is in agreement with the work of earlier researchers where ascorbic acid content was reduced upon heat treatments. (Mazzeo et al., 2011; Pellegrini et al., 2010; Yamaguchi et al., 2001).

The effect of cooking methods on DPPH scavenging and reducing potential of cocoyam was presented (Figures 1 and 2 respectively). In this study cooking methods significantly increased the reducing potential of the cocoyam extracts and also its DPPH scavenging ability. The results revealed that the extracts were able to scavenged DPPH in a manner that is concentration dependent. Based on the IC_{50} presented in Table 2, there was significant difference ($P< 0.05$) in their ability to scavenge DPPH. Roasted sample has significantly ($P< 0.05$) enhanced DPPH activity when compared with boiled and raw samples. The ferric reducing power was based on the ability of the sample to reduce the transition metal Fe^{3+} by electron transfer to Fe^{2+}. A

higher activity is indicated by higher absorbance at 700 nm. The ability of the cocoyam samples to reduce the Fe^{3+} to Fe^{2+} was dose dependent and significantly upon heat treatment. The ability was significantly increased in roasted sample than boiled sample. The enhanced DPPH scavenging and ferric reducing abilities of roasted cocoyam are due to increased polyphenol contents. This is in agreement with the work of Karigidi et al. (2018).

The result of lipid peroxidation inhibition (*in vitro*) in pancreas was presented in Figure 3. The result showed that the samples inhibited lipid peroxidation in a concentration dependent manner and the ability of the samples to inhibit peroxidation of lipid was significantly enhanced in thermal treated samples. Based on the IC_{50} there was no significant difference in boiled and raw samples but roasted sample was significantly enhanced when compared with the raw sample. The induction of lipid peroxidation in pancreas is by the pro-oxidant effect of Iron (Fe). This metal induces the production of lipid peroxides by stimulation of the oxidative machinery (OH) through Haber-Weiss reaction (Repetto and Boveris 2012). The extent of lipid peroxides was evaluated by the malondialdehyde formed.

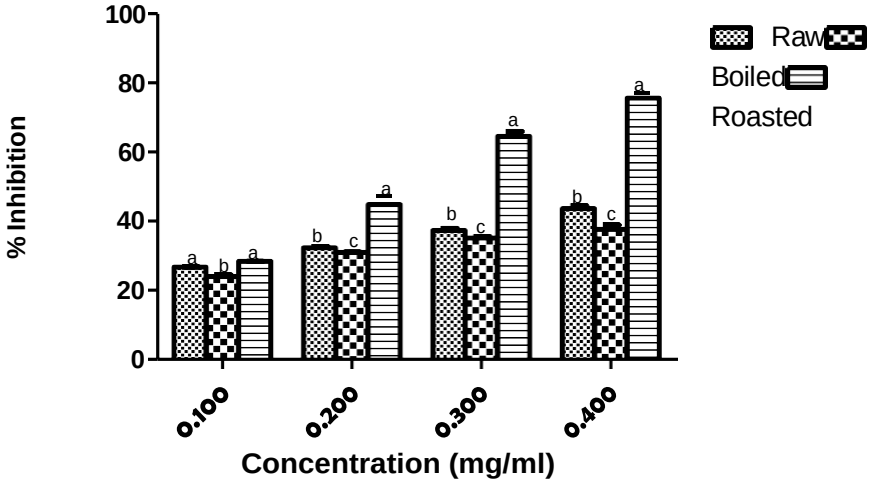

Figure 1: DPPH scavenging activity of thermal processed *Colocasia esculentum.* Values with the different superscript letter on grouped bars are significantly (P < 0.05) different.

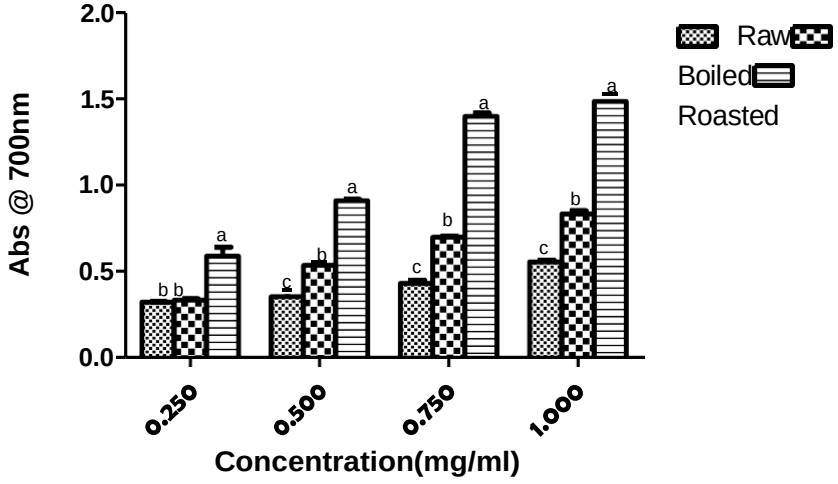

Figure 2: Reducing power of thermal processed *Colocasia esculentum.*Values with the different superscript letter on grouped bars are significantly (P < 0.05) different.

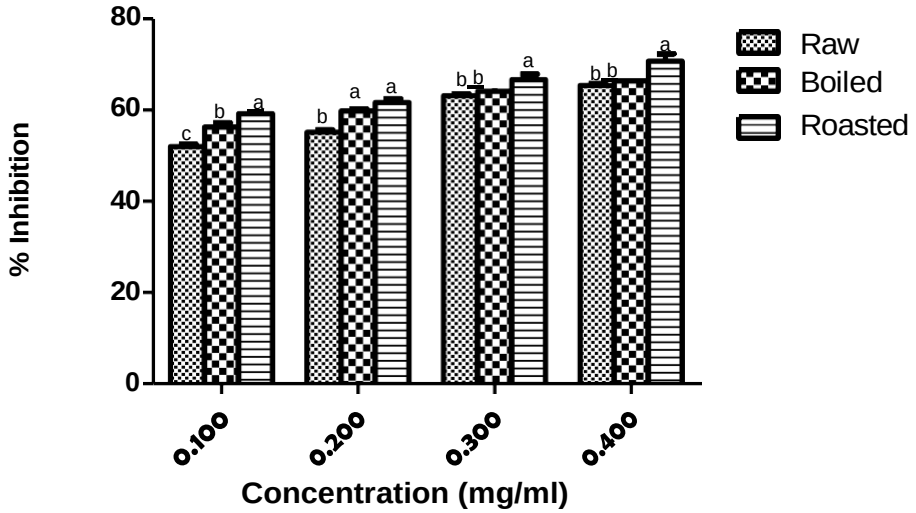

Figure 3: *In vitro* lipid peroxidation inhibitory activity of thermal processed *Colocasia esculentum.* Values with the different superscript letter on grouped bars are significantly (P < 0.05) different.

In this study, thermal treatments of cocoyam samples increased its ability to inhibit lipid peroxides in pancreas homogenate. The reduction was not significant in boiled sample but roasted sample. The enhanced inhibitory power might not be unconnected with the changed profile of polyphenol during the thermal treatment. The data obtained in this study is in agreement with the work of Lo-Scalzo and colleagues (2010) where thermal treatment enhanced the inhibitory power of eggplants via enhancement of phenolic compounds. The result for the inhibitions of α-amylase and α-glucosidase enzymes was present in Figures 4 and 5 respectively. The result showed a dose dependent inhibition which was significantly difference in the treatment groups. The IC_{50} value for these enzymes presented in Table 2 showed that inhibition was significantly increased in roasted sample and decreased in boiled sample when compared with raw sample.

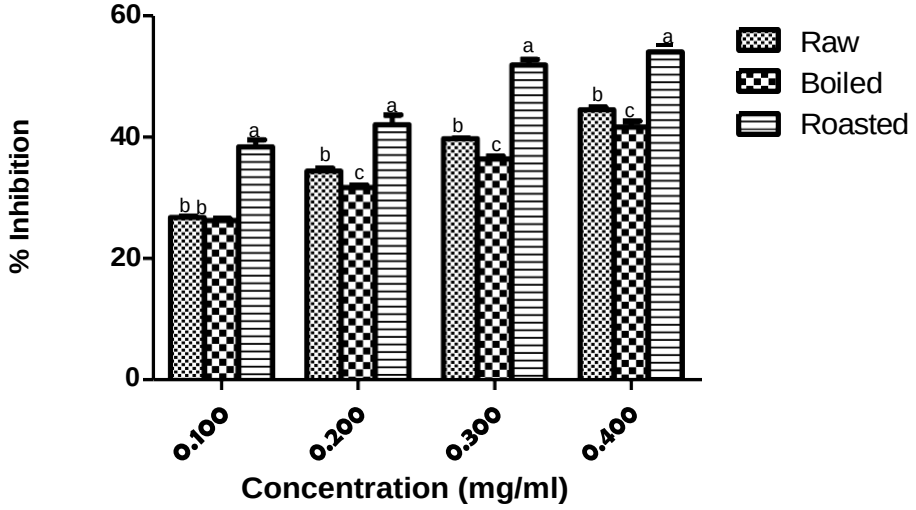

Figure 4: α-amylase inhibitory activity of thermal processed *Colocasia esculentum.*Values with the different superscript letter on grouped bars are significantly (P < 0.05) different.

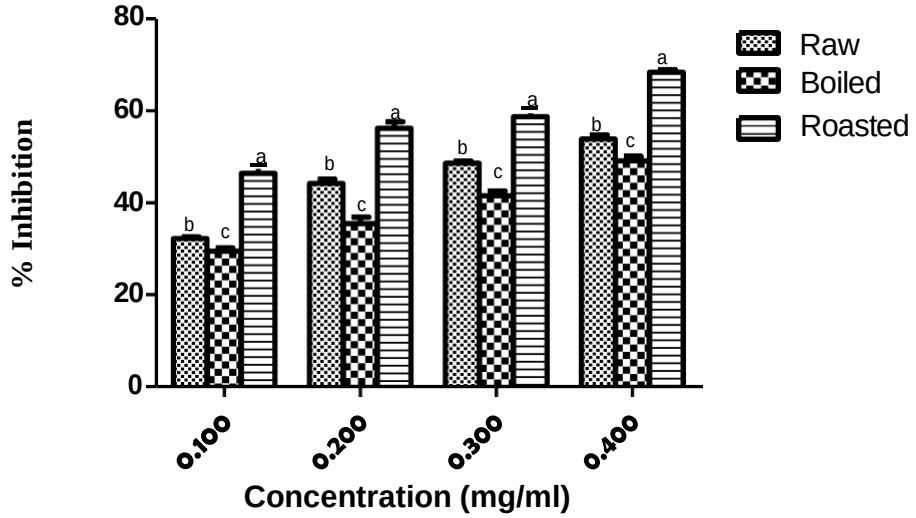

Figure 5: α-glucosidase inhibitory activity of thermal processed *Colocasia esculentum*. Values with the different superscript letter on grouped bars are significantly (P < 0.05) different.

Diabetes mellitus is fast becoming a global health concern and oxidative stress in pancreas has been implicated as one of the factors causing insulin deficiency and insulin intolerance (Snehal et al., 2017). Carbohydrate metabolizing enzymes, α-glucosidase and α-amylase, catalyze the breakdown of starch into glucose thereby increasing blood sugar concentration after a meal. The inhibitors of these enzymes are blockers which affect the activity of these enzymes thereby playing an important role in reduction of blood glucose in the body (Randhir et al., 2008). In this study, the activities of these enzymes were affected by the thermal treatments. The ability of cocoyam to inhibit these enzymes was reduced upon boiling in water while it was increased in the roasted sample. The alteration in these enzymes activity might not be unconnected with their polyphenol content. Our result is in agreement with many researchers who have reported positive correlation between polyphenol contents and inhibition of these enzymes (Kim et al., 2008; Stern et al., 2009).

Table 2: IC_{50} (mg/ml) of raw, boiled and roasted samples on DPPH, α-amylase, α-glucosidase and lipid peroxidation

	DPPH	Lipid-peroxidation	α-amylase	α-glucosidase
RC	0.450 ± 0.02^{b}	0.100 ± 0.01^{a}	0.480 ± 0.01^{b}	0.370 ± 0.02^{b}
BC	0.510 ± 0.01^{a}	0.094 ± 0.01^{a}	0.590 ± 0.04^{a}	0.420 ± 0.02^{a}
RSC	0.240 ± 0.01^{c}	0.080 ± 0.01^{b}	0.280 ± 0.02^{c}	0.170 ± 0.01^{c}

Values represent Mean± SD. Values with different letter across the column are significantly (P < 0.05) different. RC: Raw Cocoyam, BC: Boiled Cocoyam and RSC: Roasted Cocoyam

Table 3: Correlations among TPC, TFC and DPPH scavenging activity and inhibition of enzymes of carbohydrates metabolism

	TPC	TFC	DPPH	α-amylase	α-glucosidase
TPC	1				
TFC	0.888**	1			
DPPH	0.940**	0.717*	1		
α-amylase	0.965**	0.798**	0.982**	1	
α-glucosidase	0.957**	0.742*	0.992**	0.977**	1

**. Correlation is significant at the 0.01 level
*. Correlation is significant at the 0.05 level

4. CONCLUSION

This work has shown that roasting of cocoyam (*Colocasia esculenta* L. Schott) enhanced the polyphenol, antioxidant and inhibition of enzymes activity linked to diabetes mellitus. The enhancement might be due to formation of other phenolics apart from the endogenous ones, the browning pigments formed in roasted sample or mobilization of bound polyphenolic from the breakdown of cellular components and cell walls during roasting.

Conflict of interest

There is no conflict of interest.

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