

The Effect of *in vitro* Enzyme Digestion on Antioxidant and Anticholinesterase Potential of Tomato (*Lycopersicon esculentum*) Fruit and Two Commercially Processed Tomato Pastes

SULE O. SALAWU^{a*, b}, OLATUNDE F. FALOYE^a, BUKOLA B. OLA-SALAWU^b, AND AKINTUNDE A. AKINDAHUNSI^a

^a Department of Biochemistry, Federal University of Technology, Akure, Ondo State, Nigeria

^b Department of Biochemistry, Osun State University, Osogbo, Osun State, Nigeria

^c Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria

*Corresponding author

sosalawu@futa.edu.ng

TEL: +234 8132 595 807

Received: 10 September 2018; Published online: 18 January 2020

Abstract

Tomato is a horticultural crop of interest, that is widely consumed fresh or as processed products. The present investigation was to evaluate the antioxidant indices (total phenolic content, flavonoid content, ferric reducing antioxidant power, radical scavenging activities, inhibitory action against lipid oxidation) and anti-cholinesterase action (acetylcholinesterase and butyrylcholinesterase) of tomato fruits (ripe and unripe) and pastes (*paste 2* and *paste 1*) after simulated gastrointestinal digestion. The total phenolic content (mg/g GAE) of the *in vitro* digested tomato fruits and pastes showed higher values (ripe tomato: 61.08; tomato paste1: 56.02; tomato paste 2: 60.36; unripe tomato: 38.97) than the ethanolic extracts, with digested ripe tomato ranking higher. Similar results were also obtained for total flavonoid content, ferric reducing antioxidant power, and the radical scavenging activities (DPPH*, ABTS^{·+}, NO*, OH*), with the *in vitro* digested samples ranking high. The ability of the enzyme digested and ethanolic extracts of tomato fruits and pastes to inhibit iron and sodium nitroprusside induced lipid oxidation in rat's liver and brain homogenate increased in a concentration dependent manner, with the enzyme digested tomato fruits and pastes ranking high. Similarly, the ability of the *in vitro* digested tomato fruit and pastes to enhance activities of the antioxidant enzymes (GPx, GSH, SOD and Catalase) and to inhibit the formation of cholinesterases ranked high. The result of this investigation showed that the studied tomato fruit and pastes possess antioxidant and anti-cholinesterase activities that would be bio-available after the gastrointestinal digestion and by implication could be harnessed as functional food.

Keywords: *in vitro* Digestion; Antioxidant activities; Anticholinesterase Potential; Tomato fruit; Commercially- processed Tomato pastes

1 Introduction

Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals, such as superoxide, peroxy radicals, hydroxyl

radicals, nitric oxide and lipid peroxy, which process, prevents or delays damage to the cells and tissues (Birben, Sahiner, Sackesen, Erzurum, & Kalayci, 2012; Kong & Lin, 2010). Their

mechanisms of action include scavenging of reactive oxygen and nitrogen free radical species; decreasing the localized oxygen concentration, and therefore reducing molecular oxygen's oxidation potential; metabolizing lipid peroxides to non-radical products; and chelating metal ions to prevent the generation of free radicals (Barzegar, 2012; Madhuri, Qairunnisa, Suresh, Kondam, & Chandrasekhar, 2014).

A number of studies have established that some fruits, grains and vegetables have antioxidant capacity; this has been attributed principally to their polyphenol and flavonoid contents (Oboh & Ademosun, 2011; Omoba, Dada, & Salawu, 2015; Saidu & Garba, 2011; Salawu et al., 2016; Salawu, Bester, & Duodu, 2014; Yafang, Gan, & Jinsong, 2011). Regular intake of antioxidant-containing foods can reduce the risk of many chronic diseases, such as cardiovascular diseases, heart diseases, diabetes, obesity and certain cancers, and improve the endothelial function and reduce blood pressure (Pellegrino, 2016; Zhang et al., 2015). Phenolic compounds have been reported by many researchers to be present in cereals, fruit and grain crops (Salawu et al., 2014; Shahidi & Chandrasekara, 2013).

Tomatoes are a concentrated source of phenolic compounds, such as flavonoids and hydroxycinnamic acid derivatives; containing 98% of the total flavonols in tomato skin as conjugated forms of quercetin and kaempferol (C., F., H., & Didier, 2011; Hossain, Strezov, Chan, & Nelson, 2010; Skrovankova, Sumczynski, Mlcek, Jurikova, & Sochor, 2015). The high content of these compounds in tomato has gained interest due to their apparent multiple biological effects, including free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Vallverdu-Queralt et al., 2011). Among the most prominent phytochemicals in tomatoes are the carotenoids, of which lycopene is most abundant in the ripened fruit, accounting for approximately 80-90% of the total pigments (Violeta, Trandafir, & Ionica, 2013). Aside lycopene, tomatoes also contain α -, β -, γ -, δ -carotene, zeaxanthin and lutein and also neurosporene, phytoene, and phytofluene C. et al. (2011). Lycopene has the capacity to prevent free radical damage to cells caused by reactive

oxygen species. Studies have shown that it reduces the susceptibility of lymphocyte DNA to oxidative damage, inactivates H_2O_2 and NO and protects cells from NO induced membrane damage and cell death (Lobo, Patil, Phatak, & Chandra, 2010; Uttara, Singh, Zamboni, & Mahajan, 2009). Moreover, lycopene is also the most efficient singlet oxygen quencher with a capacity found to be more than twice of β -carotene (Shi, Dai, Kakuda, Mittal, & Xue, 2008). On the other hand, β -carotene is important due to its pro-vitamin A activity. Apart from carotenoids, tomato is also a source of ascorbic acid, which is an effective scavenger of superoxide, hydrogen peroxide, singlet oxygen and other free radicals (Yafang et al., 2011).

The cholinergic hypothesis of Alzheimer's disease (AD) holds that the degeneration of neurons in the basal forebrain, the associated loss of cholinergic neurotransmission in the cerebral cortex and hippocampus contribute significantly to cognitive deterioration in AD (Craig, Hong, & McDonald, 2011). The loss of cholinergic neurons in AD leads to a reduction in the synthesis of the neurotransmitter, acetylcholine (ACh), which has been associated with cognitive functions. This hypothesis has prompted the search for ways to increase ACh in AD patients. There is a need to inhibit the activity of cholinesterases (ChE) to increase the concentration of ACh needed for cognitive functions. Extracts from some plants such as *Lavandula viridis*, and some Nigeria green leafy vegetables have been documented to have ChE inhibitory activities (Costa, Gonçalves, Valentão, Andrade, & Romano, 2013; Oboh & Ademosun, 2011).

Antioxidants have to be present in some amount in the specific tissue or organ of plant foods to elicit their potential biological properties (Kasote, Katyare, Hegde, & Bae, 2015). However, the release of antioxidants from complex food materials during digestion will determine their actual biological properties. Thus, the biological extraction of antioxidants within the digestive system might be different from the extracts obtained using organic solvents. There have been reports that antioxidant activity from the chemical extracts of the food material might misjudge the actual antioxidant capacity in the digestive tract (Bhatt & Patel, 2015). *In vitro* digestion

method measures the bioavailability of the nutrient, which is the amount of the nutrient liberated from the food material during gastrointestinal digestion, which is, in turn, available for absorption in the body (Chandrasekara & Shahidi, 2012). Therefore, it can be used to evaluate a large number of food systems, which would be costly to analyze for different parameters using human or animal models.

Therefore, the objective of this work is to evaluate the antioxidant and anticholinesterase action of a Nigerian tomato fruit (Early girl) and two different commercially processed tomato paste after a simulated human gastrointestinal digestion.

2 Materials and Methods

2.1 Chemicals

Follin-Ciocalteu's reagent, gallic acid, sodium carbonate, iron (iii) chloride, potassium ferricyanide, trichloroacetic acid, aluminium chloride, potassium acetate, 2,2-diphenyl-1-picrylhydrazyl radical and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), sodium nitroprusside, sulphanilamide, N-(1-Naphthyl-ethyl-diamine-dihydrochloride), orthophosphoric acid, sodium azide, adrenalin, GHS, xanthine oxidase, xanthine, acetylcholine iodide, butrylcholine iodide were obtained from Sigma chemical company, USA. All other chemicals were obtained from standard chemical suppliers and were of analytical grade, while the water used was glass distilled.

Sample Collection

Tomato fruit, 'Early girl' (ripe and unripe) and two different types of processed tomato paste (Paste 1 and Paste 2) were bought from Shasha market in Akure, Nigeria. The fresh tomato fruit (ripe and unripe) were identified and authenticated in the Department of Crop, Soil and Pest Management, of the Federal University of Technology, Akure, Nigeria.

2.2 Sample Treatments and Preparation

The fresh tomatoes were washed and blended into paste. Both the blended and the commercially processed tomatoes were freeze-dried at the Central Laboratory of Obafemi Awolowo University, Ile-Ife, Nigeria. The freeze-dried samples (ripe tomato fruit, unripe tomato fruit, Paste 1 tomato, Paste 2 Tomato) were divided in to two groups each; the first group was extracted with ethanol, while the second group was subjected to *in vitro* enzyme (α - amylase, pepsin, pancreatin) digestion.

Preparation of Ethanolic Extract

The extraction steps were carried out by soaking 2 g of each of the lyophilized sample in 40ml of 96% ethanol for 24 hours, after which the supernatant was filtered with filter paper No.42 and stored in an amber bottle. This process was repeated by the addition of another 40ml of 96% ethanol to the residue for another 24 hours and the supernatant pulled together. The filtrate was stored at 4°C.

Preparation of Brain and Liver Homogenates for Lipid Peroxidation Assay

The rats were anesthetized with chloroform and then sacrificed. The cerebral tissue (whole brain) and liver were rapidly isolated, weighed and placed on ice. The tissues were subsequently homogenized in 0.1 M Tris-HCl pH 7.4 with about ten up and ten down strokes at approximately 1,200 rpm in a Teflon-glass homogenizer. The homogenate was centrifuged for 10 minutes at 3,000 g to yield a pellet that was discarded and the low-speed supernatant was kept for lipid peroxidation assay, acetylcholinesterase (AChE) and butrylcholineesterase (BuChE) inhibition assay.

2.3 *In vitro* Enzymatic Digestion

The *in vitro* digestion using sequential enzymatic steps is based on a slightly modified method reported by Ross, Gutierrez-Botero, and Van Am-

burgh (2013). Two gram of each freeze-dried tomato sample was weighed and dissolved in 40 mL of distilled water. 300 μ L of α -amylase (32.5 mg of α -amylase was dissolved in 25 mL of 1 mM calcium chloride at pH 7) was added to the tubes. The tubes were incubated in a shaking water bath set at 37°C for 10 minutes and at 80 strokes/minute. After 10 minutes, the pH was adjusted to 2 using concentrated HCl. After 30 minutes of incubation in a shaking water bath set at 37 °C, 2 mg pepsin, which was dissolved in 1 mL of 0.05 M HCl, was added to the tube. The tubes were then incubated in a shaking water bath set at 37 °C for 10 minutes and at 80 strokes/minute.

After further 20 minutes of shaking the tubes, the pH was adjusted to 6 using NaOH. Then, 10 mL of pancreatin (3 g of pancreatin was dissolved in 20 mL distilled water) was added, and the tubes were incubated in a shaking water bath set at 37 °C for 20 minutes. The pH was adjusted finally to 7.5 using NaOH (simulating the pH conditions in the small intestine). Then, the tubes were incubated for 10 minutes in a shaking water bath set at 37 °C. The digested sample was incubated at 100 °C for 4 minutes to inactivate the enzymes, and the digested sample was then centrifuged for 60 minutes at 3,200 g, and then, the soluble fraction was kept in the refrigerator for antioxidant, anticholinesterase and lipid peroxidation analyses. The insoluble fraction was discarded. An undigested control was also prepared using the same scheme of *in vitro* digestion but without the enzymes.

2.4 Antioxidant Indices

A modified Folin–Ciocalteu method (Berker, Olgun, Ozyurt, Demirata, & Apak, 2013) and a method reported by Meda, Lamien, Romito, Millogo, and Nacoulma (2005) were used to measure the total phenolic content (TPC) and total flavonoid content (TFC), respectively. Centrifuged ethanol extracts and enzyme digest were reacted with Folin–Ciocalteu phenol reagent and sodium carbonate (20 per cent, w/v for 2 hours) and absorbance was read at 760 nm. Gallic acid was used as a standard and the TPC expressed as mg of gallic acid equivalent (GAE) per gram.

Similarly, the centrifuged ethanol extracts and enzyme digests were reacted with 0.5 mL ethanol, 50 μ L of 10 per cent $AlCl_3$, 50 μ L of 1 mol L⁻¹ potassium acetate and 1.4 mL water and allowed to incubate at room temperature for 30 minutes. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The TFC was calculated using quercetin as a standard by making use of a seven-point standard curve (0–100 μ g/mL). The ferric-reducing properties of the ethanolic extracts and enzyme digests were determined using the method of Oyaizu (1986), by reacting 1 mL ethanol extracts and enzyme digest with 1 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1 mL of 1 percent potassium ferricyanide. The mixture was incubated at 50 °C for 20 minutes, and then, 1 mL of 10 percent trichloroacetic acid was added. This mixture was centrifuged at 353 x g for 10 minutes. The supernatant (2 mL) was mixed with an equal volume of water and 0.4 mL of 0.1 percent ferric chloride. The absorbance was measured at 700 nm. The ferric-reducing antioxidant power was expressed as milligram of ascorbic acid equivalent/gram of the sample. Radical scavenging antioxidant activity of the ethanolic extracts and enzyme digests were determined using the 2, 2–azino-bis-3-thylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay according to Awika, Rooney, Wu, Prior, and Cisneros-Zevallos (2003); 1, 1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to the method of Brand-Williams, Cuvelier, and Berset (1995); Hydroxyl radical scavenging assay by Halliwell and Gutteridge (1999) and nitric oxide radical scavenging assay by Griess reaction using the method of Sangameswaran, Balakrishnan, Deshraj, and Jayakar (2009). Trolox was used as a standard for ABTS and DPPH anti-radical assay, and the values reported as μ mol trolox equivalent anti-oxidant capacity per gram (μ mol TE/g). Ascorbic acid was used as the standard for nitric oxide radical assay and the values were expressed as percentage inhibition.

2.5 Lipid Peroxidation Assay

The ability of the ethanolic extract and the *in vitro*-digested samples to inhibit lipid peroxida-

tion was assessed using a modified method presented in the study by Ohkawa, Ohishi, and Yagi (1979). The homogenates obtained (100 μL) from white rat's liver and brain were incubated with (or without for the blank); 50 μL of freshly prepared 0.071 mM FeSO_4 , 30 μL 100 mM Tris-HCl (pH 7.4) and ethanol extract/*in vitro*-digested samples (0-100 μL), together with an appropriate volume of deionized water, to give a total volume of 300 μL were then incubated at 37 °C for 1 hour. The color reaction was carried out by adding 300 μL of 8.1 percent w/v sodium dodecyl sulphate, 500 μL of 0.15 percent v/v acetic acid solution (pH 3.4) and 500 μL of 0.6 percent w/v thiobarbituric acid. The absorbance was read after cooling the tubes at a wavelength of 532 nm. As control, the homogenate was peroxidized with 0.071 mM FeSO_4 without the *in vitro* enzyme-digested samples or ethanolic extracts. A blank containing other reagents except FeSO_4 , homogenate and the extracts was also prepared.

2.6 Acetylcholine and Butrylcholine Esterase Inhibitory Activity Assay

AChE and BuChE inhibitory activity were measured by the spectrophotometric method developed by Lin, Liu, Lin, and Wu (2004) with slight modifications, using acetylcholine iodide and butrylcholine iodide as substrates, respectively. The rate of production of thiocholine is determined by the continuous reaction of the thiol with 5, 5-dithiobis-2-nitrobenzoate (DTNB) ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid. Briefly, 1 mL of 10 mM DTNB dissolved in 10 mM sodium phosphate buffer (pH 7.0) was added to 0.6 mL of distilled water. The brain homogenate, acting as the enzyme source (0.1 mL) and the digested sample/ethanol extract (0.1 mL) was then added to the mixture and incubated for 2 minutes at 25 °C before 0.2 mL of 8mM acetylcholine iodide (substrate) was added. The absorbance of the mixture was read at 412 nm at intervals of 30 seconds for 5 minutes immediately after the substrate was added. For the control, 0.1 mL of brain homogenate (enzyme source) was added to 1mL of 10 mM DTNB dis-

solved in 10 mM sodium phosphate buffer (pH 7.0) and 0.7 mL of distilled water. The mixture was incubated at 25 °C for 2 minutes before 0.2 mL of 8 mM of acetylcholine iodide was added and the absorbance was taken immediately. The distilled water (0.1 mL) and 10 mM DTNB (1 mL) were used as blank. The procedure was repeated using 8 mM butrylcholine iodide as the substrate. The results were expressed in $\mu\text{mol min}^{-1}\text{mg protein}^{-1}$ using a molar extinction coefficient of $13.6 \times 10^3 \text{ M}^{-1}\text{cm}^{-1}$.

2.7 Statistical Analysis

All the analyses were run in triplicates. Results were then computed using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and followed by one-way Anova Tukey Multiple Range Test (TMRT) to compare the means that showed significant variation by using graph pad for windows. The significance level was set at $p < 0.05$.

3 Results and Discussions

3.1 Results

Antioxidant Indices

The result of the total phenolic content (TPC), is as presented on Table 1. The results revealed a higher TPC (mg GAE/g sample) for all *in vitro* enzyme-digested tomato samples (ripe tomato: 61.08; processed tomato *paste 2*: 60.36; processed tomato *paste 1*: 56.02; unripe tomato: 38.97) than the ethanolic extracts (ripe tomato: 35.59; processed tomato *paste 2*: 30.28; processed tomato *paste 1*: 29.99; unripe tomato: 14.91). Thus, the maximum amount of total phenolic compounds was released during *in vitro* digestion process because of the activity of the enzymes (α -amylase, pepsin and pancreatin) of the gastrointestinal tract. The result further showed that the TPC of *in vitro* digested ripe tomato (61.08 mg GAE g^{-1} sample) was higher than *in vitro* digested unripe sample (38.97 mg GAE g^{-1} sample) and that TPC of ripe unprocessed tomato was higher than the processed tomato (both *paste 2* and *paste 1*).

The total flavonoid content (mg quercetin equivalent/g of sample) of ethanolic extracts and *in vitro* enzyme digested samples of tomato fruits (ripe and unripe) and commercially processed tomato *paste 2* and *paste 1* is as shown in Table 1. The result also showed that the *in vitro* enzyme digested samples have higher total flavonoid content (ripe tomato: 50.86; processed tomato *paste 2*: 43.95; processed tomato *paste 1*: 29.47; unripe tomato: 25.06) than the ethanolic extracts (ripe tomato: 21.25; processed tomato *paste 2*: 18.06; processed tomato *paste 1*: 10.62; unripe tomato: 10.01). The result also revealed that processed tomato *paste 2* has higher TFC compared to *paste 1*.

Similarly, the result of ferric reducing antioxidant power (mg ascorbic acid equivalent/g of sample) of ethanolic extracts and, *in vitro* enzyme digested samples of tomato fruits (ripe and unripe) and commercially processed tomato *paste 2* and *paste 1* (Table 1), revealed that the digested samples have higher reducing power (ripe tomato: 19.89; processed tomato *paste 2*: 18.70; processed tomato *paste 1*: 12.86; unripe tomato: 10.36) than the ethanolic extracts (ripe tomato: 11.98; tomato *paste 2*: 11.40; tomato *paste 1*: 7.39; unripe tomato: 1.70). Similar to what was observed in the total phenolic and flavonoid estimation, *in vitro* digested ripe tomato showed higher reducing power than digested unripe tomato. In addition, higher reducing power was recorded in ripe tomato compared to the processed tomato in both ethanolic extracts and digested samples.

The result of the radical scavenging potentials of the *in vitro* enzyme digested tomato samples displayed a higher radical scavenging activities (DPPH^{*}, ABST⁺, NO^{*} and ·OH^{*}) compared to the ethanolic extracts. In addition, radical scavenging activities (DPPH[·], ABTS⁺, NO[·] and ·OH) was higher in digested ripe tomato than in digested processed tomato, while processed tomato *paste 2* revealed higher radical scavenging activities than tomato *paste 1*.

Antioxidant Enzyme Assay

In this current study, the ethanolic extracts and *in vitro* digested samples revealed enhanced activities of the antioxidant enzymes (GPx, GSH,

SOD and CAT) in a manner similar to the trends observed in the phenolic content estimation and radical scavenging activities 3. The activities of the enzymes were enhanced to a higher degree after simulated *in vitro* digestion of both the tomato fruit and commercially processed tomato product. The digested ripe tomato fruit reveal higher activities of the antioxidant enzyme compared to processed tomato paste and unripe tomato fruit. Tomato *paste 2* also showed higher enhanced activities of the enzymes than tomato *paste 1*.

Lipid Peroxidation Assay

Both the ethanolic extracts and *in vitro* digested samples of tomato (fruit and paste) showed potential for inhibition of lipid peroxidation 2. Similarly, *in vitro* digested samples revealed higher inhibitory action against lipid oxidation compared to ethanolic extracts. Higher inhibitory action was also recorded for digested ripe tomato while the least inhibition action was observed in unripe tomato. The result also showed that processed tomato *paste 2* has higher inhibition action than tomato *paste 1*.

Acetylcholine and Butrylcholine Esterase Inhibitory Activity Assay

The result obtained from the cholinesterase (AChE and BuChE) inhibitory action of tomato fruit and paste revealed that *in vitro* enzyme digested and ethanolic extracts of tomato fruit and paste samples possessed appreciable potential of inhibiting AChE and BuChE activity (Figures 1 and 2). The *in vitro* digested samples displayed higher inhibitory action compared to the ethanolic extracts. The highest % inhibitory action against AChE and BuChE activity was recorded for digested ripe tomato followed by tomato *paste 2*, while the least % inhibition was observed in unripe tomato. Furthermore, processed tomato *paste 2* tomato had higher inhibition potential than processed tomato *paste 1*.

3.2 Discussion

The observed higher phenolic content in the *in vitro* digested tomato samples is in agreement

Table 1: Antioxidant Indices of tomato fruit (ripe and unripe) and paste (*paste 2* and *paste 1*)

Samples	TPC (mg GAE/g)	TFC (mg QE/g)	FRAP (mg AAE/g)	DPPH ($\mu\text{mol TE/g}$)	ABTS ($\mu\text{mol TE/g}$)	NO (% Inhibition)	OH (% Inhibition)
RT	35.59 \pm 0.09 ^d	21.25 \pm 1.01 ^c	11.98 \pm 0.01 ^d	50.03 \pm 0.40 ^d	89.86 \pm 0.00 ^c	58.67 \pm 0.60 ^c	18.11 \pm 0.90 ^d
UT	14.91 \pm 0.05 ^a	10.01 \pm 0.35 ^a	01.70 \pm 0.01 ^a	34.14 \pm 0.20 ^a	83.40 \pm 0.40 ^a	48.36 \pm 0.60 ^a	0.47 \pm 0.18 ^a
P1	29.99 \pm 0.03 ^b	10.62 \pm 0.62 ^a	07.40 \pm 0.00 ^b	37.04 \pm 0.35 ^b	87.64 \pm 0.60 ^b	49.24 \pm 0.11 ^a	10.57 \pm 2.09 ^c
P2	30.28 \pm 0.05 ^c	18.06 \pm 0.30 ^b	11.40 \pm 0.21 ^d	42.13 \pm 0.12 ^c	89.38 \pm 0.60 ^c	53.48 \pm 0.00 ^b	10.60 \pm 0.09 ^c
DRT	61.08 \pm 0.30 ^{f,g}	50.86 \pm 0.41 ^g	19.89 \pm 0.00 ^g	71.90 \pm 0.63 ^g	95.49 \pm 0.14 ^e	69.06 \pm 0.51 ^f	35.71 \pm 0.18 ^g
DUT	38.97 \pm 0.55 ^e	25.06 \pm 0.00 ^d	10.36 \pm 0.00 ^e	50.75 \pm 0.81 ^d	89.86 \pm 0.14 ^c	60.75 \pm 0.17 ^d	06.99 \pm 0.73 ^b
DP1	56.02 \pm 0.05 ^f	29.47 \pm 0.66 ^e	12.86 \pm 0.02 ^e	63.34 \pm 0.37 ^e	93.75 \pm 0.00 ^d	64.67 \pm 0.37 ^e	26.87 \pm 0.73 ^e
DP2	60.36 \pm 0.35 ^{f,g}	43.95 \pm 0.66 ^f	18.70 \pm 0.01 ^f	65.06 \pm 0.27 ^f	94.86 \pm 0.60 ^e	67.55 \pm 0.06 ^f	32.43 \pm 0.91 ^f

Values represent mean \pm standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly ($p < 0.05$). Abbreviations: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes; GAE=Gallic Acid Equivalent; QE=Quercetin Equivalent; AAE=Ascorbic Acid Equivalent; TE= Trolox equivalents.

Table 2: IC50 values of ethanolic extract and *in vitro* digested tomato fruit and paste on iron and sodium nitropruside induced lipid oxidation (mg/g)

Samples	Fe ²⁺ induced		SNP induced	
	Brain	Liver	Brain	Liver
RT	108.3 \pm 0.02 ^d	70.00 \pm 0.29 ^d	108.0 \pm 1.01 ^d	60.91 \pm 0.43 ^b
UT	183.2 \pm 0.44 ^h	119.6 \pm 1.34 ^h	183.2 \pm 0.19 ^h	125.3 \pm 1.63 ^g
P1	174.6 \pm 0.16 ^g	78.42 \pm 0.34 ^f	124.6 \pm 0.51 ^g	80.49 \pm 1.56 ^e
P2	161.3 \pm 0.08 ^f	72.47 \pm 1.02 ^e	116.2 \pm 1.56 ^e	65.05 \pm 0.00 ^c
DRT	69.51 \pm 0.09 ^a	53.21 \pm 0.45 ^a	79.51 \pm 0.00 ^a	52.01 \pm 0.04 ^a
DUT	126.1 \pm 1.02 ^e	95.09 \pm 0.69 ^g	122.1 \pm 0.97 ^f	86.54 \pm 1.02 ^f
DP1	83.13 \pm 0.06 ^c	66.19 \pm 0.75 ^c	83.13 \pm 0.04 ^b	76.81 \pm 0.56 ^d
DP2	76.42 \pm 0.05 ^b	63.73 \pm 1.34 ^b	94.42 \pm 0.68 ^c	61.01 \pm 1.01 ^b

Values represent mean \pm standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly ($p < 0.05$). Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes

Table 3: Effect of ethanolic extract and *in vitro* digested samples of tomato fruit and paste on antioxidant enzyme activities

Samples	GSH (U/mL)	GPX (U/mL)	SOD (U/mL)	CATALASE (U/mL)
RT	8.54±0.65 ^c	14.6±0.18 ^e	3.14±0.02 ^c	2.49±0.09 ^c
UT	5.39±0.26 ^a	1.68±0.11 ^a	0.68±0.13 ^a	1.10±0.02 ^a
P1	6.43±0.16 ^b	2.83±0.9 ^b	1.64±0.03 ^b	1.57±0.11 ^b
P2	6.63±0.08 ^b	8.38±0.06 ^d	3.12±0.04 ^c	2.15±0.47 ^b
DRT	10.68±0.50 ^e	34.3±0.02 ^h	6.04±0.02 ^e	5.15±0.14 ^d
DUT	5.78±0.65 ^a	6.62±1.98 ^c	1.46±0.10 ^b	1.11±0.01 ^a
DP1	9.24±0.17 ^d	17.30±0.75 ^f	3.27±0.00 ^c	2.61±0.23 ^b
DP2	9.94±0.13 ^d	22.87±0.08 ^g	4.06±0.02 ^d	2.67±0.25 ^b

Values represent mean± standard deviation of triplicate experiments. Values with different superscripts in the same column differ significantly (p<0.05) Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Tomatoes; DUT= Digested Unripe Tomatoes; DP2= Digested Paste 2 Ripe Tomatoes; DP1= Digested Paste 1 Tomatoes; GSH= Glutathion; PX=Glutathion peroxidase; SOD=Superoxide dismutase.

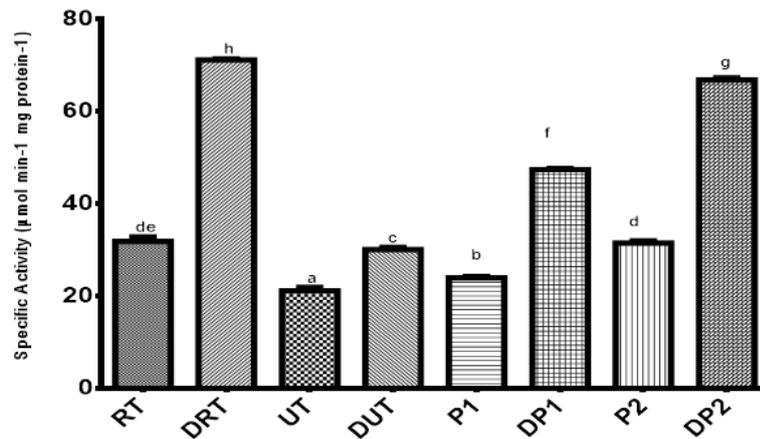


Figure 1: Acetylcholinesterase Inhibitory activity of *in vitro* digested tomato fruit and paste. Values are given as mean ± SE of independent experiments performed in triplicate. Bars with different letters are significantly different (p< 0.05) by Tukey Test. Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes

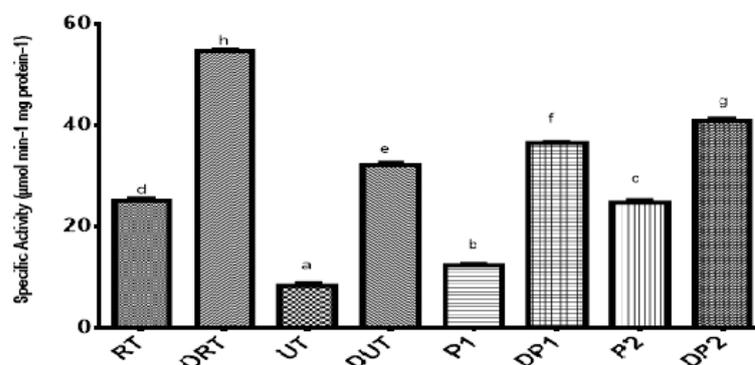


Figure 2: Butyrylcholinesterase Inhibitory of *in vitro* digested tomato fruit and paste. Values are given as mean \pm SE of independent experiments performed in triplicate. Bars with different letters are significantly different ($p < 0.05$) by Tukey Test. Abbreviation: RT= Ripe Tomatoes; UT= Unripe Tomatoes; P1= Paste 1 Tomatoes; P2= Paste 2 Tomatoes; DRT= Digested Ripe Tomatoes; DUT= Digested Unripe Tomatoes; DPT= Digested Paste 2 Tomatoes; DGT= Digested Paste 1 Tomatoes

with a previous report of Tagliazucchi, Verzeloni, Bertolini, and Conte (2010), which suggested that digestion might be a determinant factor in the release of nutritionally relevant compounds from the food matrix. The result further showed that the TPC of *in vitro* digested ripe tomato ($61.08 \text{ mg GAE g}^{-1} \text{ sample}$) was higher than that of the digested unripe sample ($38.97 \text{ mg TAE g}^{-1} \text{ sample}$). The observed higher TPC in enzyme digested ripe tomato may be associated with a considerable accumulation of lycopene in ripped tomato, which is due to the enhanced enzymatic activity of phytoene synthase I that causes a massive production of lycopene precursors in ripening tomato fruits (Fraser, Enfissi, & Bramley, 2009). The observed higher TPC of ripe unprocessed tomato compared with the processed tomato (both *paste 2* and *paste 1*), may be ascribed to the different processing methods. This is in correlation with the work of Anese, Mirolo, Beraldo, and Lippe (2013), where it was reported that non-thermal processing seems to have an adverse effect on bio-accessibility of lycopene. For example, the loss of cell integrity observed with increasing ultrasonication time was accompanied by a decrease in lycopene bio-accessibility.

The observed high flavonoid content of the *in vitro* digested tomato fruit and pastes compared

to the ethanolic extract is in agreement with the report of Salawu, Ajiboye, Akindahunsi, and Boligon (2017), where higher flavonoid content were recorded for the *in vitro* digested white and yellow bitter yams compared to their raw counterparts. This observation might be due to the breakdown of the insoluble fiber matrix of both bitter yam varieties thereby making its flavonoids more accessible for further breakdown by the enzymes of gastro intestinal tract. Ripe digested sample have a higher TFC than the digested processed samples. Ranilla, Genovese, and Lajolo (2007) mentioned that cooking time, temperature, soaking and draining can significantly reduce antioxidant activity of plants. This is also in agreement with the report of Fawole and Opara (2013), it was suggested that naturally occurring flavonoid could be obviously lost during processing and storage, which could affect the overall antioxidant and nutritional value after post-harvest treatment. The observed high flavonoid content in tomato *paste 2* compared to *paste 1* might be due to the varietal differences in the tomato fruits used in the production of tomato *paste 1* and tomato *paste 2* and the processing methods. Macheix, Fleuriet, and Billot (1990) suggested that genetic control is the primary factor in determining phenols in fruits and vegetables, their level may be affected by environmental condi-

tions, such as light, temperature and processing methods.

The ferric reducing antioxidant power (FRAP) of a compound may serve as an important indicator of its potential antioxidants activity (Liu et al., 2013). The observed higher FRAP in the *in vitro* enzyme digested samples is in agreement with the work of Saura-Calixto and Goni (2006), who reported that *in vitro* enzyme digested plant yield a higher reducing power than organic extracts. The observed high reducing power in ripe tomato for both ethanolic extracts and *in vitro* digested samples compared to commercially processed tomato pastes may be attributed to various processing steps of the tomato paste. This is in agreement with the report of Szydłowska-Czerniak, Trokowski, Karlovits, and Szlyk (2011), where it was reported that ferric reducing antioxidant power was decreased by 41% during thermal processing of palm oil.

Most plant foods are rich sources of free radical scavenging molecules and other metabolites, which are rich in antioxidant activity (Nimse & Pal, 2015). The observed high radical scavenging activities (DPPH \cdot , ABST \cdot^+ , NO \cdot and \cdot OH) the *in vitro* enzyme digested tomato samples compared to the ethanolic extracts is in agreement with the work of Hachibamba, Dykes, Awika, Minnaar, and Duodu (2013), where it was found that the TPC and radical scavenging properties of cowpea was increased with simulated *in vitro* enzyme digestion. The observed high radical scavenging activities in digested ripe tomato fruits compared to the commercially processed tomato pastes is likely to be as a result of heat processing to attain final solid level. Longer processing times, required to achieve the desired final solids levels, may be associated with increased losses. Other studies have also shown thermal processing to decrease significantly the TPC, anthocyanin content, and antioxidant activity (Hiemori, Koh, & Mitchell, 2009).

It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or

antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more palpable (Worthington Enzyme Manual, 2009). The enhanced antioxidant enzyme parameters in the presence of the ethanolic extracts and *in vitro* digested tomato samples in a manner similar to the trends observed in the phenolic content estimate and radical scavenging assays is in agreement Jalili, Ilkhanipour, Heydari, Farshid, and Salehi (2007), and Ergüder and Durak (2006). These authors, confirmed that administration of lycopene at 100, 200 and 300 mg/kg doses decreased the level of oxidant parameters (MDA) and significantly increased blood and gastric antioxidant parameters (SOD, CAT and GSH-Px), and suggested that lycopene may reduce oxidative injury of gastric cancer rats partly through stimulating antioxidant enzyme activities. Antioxidant enzymes are, therefore, critical for maintaining optimal cellular and systemic health and well-being.

Both the ethanolic extracts and *in vitro* digested samples of tomato (fruit and paste) showed potential of inhibiting lipid oxidation. The result correlate with the work of Luo and Wu (2011), where it was reported that lycopene treatment in rats, with induced gastric cancer significantly ameliorated an increased MDA level at the end of the experiment. Since flavonoid represent the major component of the total phenolic content in tomato (Toor & Savage, 2005), hence, the flavonoids, lycopene and other phenolic compounds present in tomato fruit and paste may be responsible for their ability to inhibit lipid peroxidation.

In recent years, the search for inhibitors of cholinesterases has grown in interest, since these enzymes are associated with Alzheimer's disease, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease among others (Mukherjee, Kumar, Mal, & Houghton, 2007; Vinholes et al., 2011). Plant alkaloids are best known for inhibiting cholinesterase enzymes, however, recent reports have indicated new classes of cholinesterase inhibiting phytochemicals such as coumarins, flavonols, terpenoids, and especially monoterpenes that are relevant antioxidant phytochemicals (Katalinic, Bosak, & Kovarik, 2014; Szwajgier, 2014). Hence, the observed anti-cholinesterase action in both ethanolic extracts

and *in vitro* enzyme digest of the studied tomato samples could be ascribed to the constituent phytochemicals in tomato fruits and commercially processed tomato pastes.

4 Conclusion

The result of this investigation showed that the studied tomato fruit and pastes possess a substantial level of antioxidant and anticholinesterase activities after the simulated human gastrointestinal digestion, with the ripe tomato fruit ranking high. This by implication suggest that the health promoting phytoconstituents of the tomato fruit and pastes would be readily bio-available after passing through the gastrointestinal digestive tract and therefore could be harnessed as functional food.

References

- Anese, M., Mirolo, G., Beraldo, P., & Lippe, G. (2013). Effect of ultrasound treatments of tomato pulp on microstructure and lycopene *in vitro* bioaccessibility. *Food Chemistry*, *136*(2), 458–463. doi:[10.1016/j.foodchem.2012.08.013](https://doi.org/10.1016/j.foodchem.2012.08.013)
- Awika, J. M., Rooney, L. W., Wu, X. L., Prior, R. L., & Cisneros-Zevallos, L. (2003). Screening methods to measure antioxidant activity of sorghum (*sorghum bicolor*) and sorghum products. *Journal of Agricultural and Food Chemistry*, *51*(23), 6657–6662. doi:[10.1021/jf034790i](https://doi.org/10.1021/jf034790i)
- Barzegar, A. (2012). The role of electron-transfer and H-atom donation on the superb antioxidant activity and free radical reaction of curcumin. *Food Chemistry*, *135*(3), 1369–1376. doi:[10.1016/j.foodchem.2012.05.070](https://doi.org/10.1016/j.foodchem.2012.05.070)
- Berker, K. I., Olgun, F. A. O., Ozyurt, D., Demirata, B., & Apak, R. (2013). Modified folin-ciocalteu antioxidant capacity assay for measuring lipophilic antioxidants. *Journal of Agricultural and Food Chemistry*, *61*(20), 4783–4791. doi:[10.1021/jf400249k](https://doi.org/10.1021/jf400249k)
- Bhatt, A., & Patel, V. (2015). Antioxidant potential of banana: Study using simulated gastrointestinal model and conventional extraction. *Indian Journal of Experimental Biology*, *53*(7), 457–461.
- Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, *5*(1), 9–19. doi:[10.1097/WOX.0b013e3182439613](https://doi.org/10.1097/WOX.0b013e3182439613)
- Brand-Williams, W., Cuvelier, M., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. doi:[10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- C., R. R., F., E. S. A., H., P. S., & Didier, M. (2011). Anti-oxidant properties and other functional attributes of tomato: An overview. *International Journal of Food and Fermentation Technology*, *1*(2), 139–148. Retrieved from <https://www.indianjournals.com/ijor.aspx?target=ijor:ijfft&volume=1&issue=2&article=001>
- Chandrasekara, A., & Shahidi, F. (2012). Bioaccessibility and antioxidant potential of millet grain phenolics as affected by simulated *in vitro* digestion and microbial fermentation. *Journal of Functional Foods*, *4*(1), 226–237. doi:[10.1016/j.jff.2011.11.001](https://doi.org/10.1016/j.jff.2011.11.001)
- Costa, P., Gonçalves, S., Valentão, P., Andrade, P. B., & Romano, A. (2013). Accumulation of phenolic compounds in *in vitro* cultures and wild plants of *lavandula viridis* l'hér and their antioxidant and anticholinesterase potential. *Food and Chemical Toxicology*, *57*, 69–74. doi:<https://doi.org/10.1016/j.fct.2013.03.006>
- Craig, L. A., Hong, N. S., & McDonald, R. J. (2011). Revisiting the cholinergic hypothesis in the development of alzheimer's disease. *Neuroscience & Biobehavioral Reviews*, *35*(6), 1397–1409. doi:[10.1016/j.neubiorev.2011.03.001](https://doi.org/10.1016/j.neubiorev.2011.03.001)
- Ergüder, I., & Durak, I. (2006). Effects of computer use on human salivary oxidant/antioxidant status. *Online Journal of Biological Sciences*, *6*. doi:[10.3844/ojbsci.2006.14.17](https://doi.org/10.3844/ojbsci.2006.14.17)
- Fawole, O. A., & Opara, U. L. (2013). Effects of storage temperature and duration on physiological responses of pomegranate fruit. *Industrial Crops and Products*, *47*, 300–309. doi:[10.1016/j.indcrop.2013.03.028](https://doi.org/10.1016/j.indcrop.2013.03.028)

- Fraser, P. D., Enfissi, E. M., & Bramley, P. M. (2009). Genetic engineering of carotenoid formation in tomato fruit and the potential application of systems and synthetic biology approaches. *Archives of Biochemistry and Biophysics*, *483*(2), 196–204. Recent Achievements of Carotenoid Science and Technology. doi:[10.1016/j.abb.2008.10.009](https://doi.org/10.1016/j.abb.2008.10.009)
- Hachibamba, T., Dykes, L., Awika, J., Minnaar, A., & Duodu, K. G. (2013). Effect of simulated gastrointestinal digestion on phenolic composition and antioxidant capacity of cooked cowpea (*vigna unguiculata*) varieties. *International Journal of Food Science and Technology*, *48*(12), 2638–2649. doi:[10.1111/ijfs.12260](https://doi.org/10.1111/ijfs.12260)
- Halliwell, B., & Gutteridge, J. M. C. (1999). *Free radicals in biology and medicine*. New York: Oxford University Press.
- Hiemori, M., Koh, E., & Mitchell, A. E. (2009). Influence of cooking on anthocyanins in black rice (*oryza sativa* l. japonica var. sbr). *Journal of Agricultural and Food Chemistry*, *57*(5), 1908–1914. doi:[10.1021/jf803153z](https://doi.org/10.1021/jf803153z)
- Hossain, M. K., Strezov, V., Chan, K. Y., & Nelson, P. F. (2010). Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*lycopersicon esculentum*). *Chemosphere*, *78*(9), 1167–1171. doi:[10.1016/j.chemosphere.2010.01.009](https://doi.org/10.1016/j.chemosphere.2010.01.009)
- Jalili, S., Ilkhanipour, M., Heydari, R., Farshid, A. A., & Salehi, S. (2007). The effects of vitamin e on endosulfan-induced oxidative stress in rat heart. *Pakistan Journal of Nutrition*, *6*(4), 375–380.
- 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. doi:[10.7150/ijbs.12096](https://doi.org/10.7150/ijbs.12096)
- Katalinic, M., Bosak, A., & Kovarik, Z. (2014). Flavonoids as inhibitors of human butyrylcholinesterase variants. *Food Technology and Biotechnology*, *52*(1, SI), 64–67.
- Kong, Q., & Lin, C.-I. G. (2010). Oxidative damage to RNA: Mechanisms, consequences, and diseases. *Cellular and Molecular Life Sciences*, *67*(11), 1817–1829. doi:[10.1007/s00018-010-0277-y](https://doi.org/10.1007/s00018-010-0277-y)
- Lin, G., Liu, Y. C., Lin, Y. F., & Wu, Y. G. (2004). Ortho effects in quantitative structure-activity relationships for acetylcholinesterase inhibition by aryl carbamates. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *19*(5), 395–401. doi:[10.1080/14756360410001733694](https://doi.org/10.1080/14756360410001733694)
- Liu, Y., Pukala, T. L., Musgrave, I. F., Williams, D. M., Dehle, F. C., & Carver, J. A. (2013). Gallic acid is the major component of grape seed extract that inhibits amyloid fibril formation. *Bioorganic & Medicinal Chemistry Letters*, *23*(23), 6336–6340. doi:[10.1016/j.bmcl.2013.09.071](https://doi.org/10.1016/j.bmcl.2013.09.071)
- Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, *4*(8), 118.
- Luo, C., & Wu, X.-G. (2011). Lycopene enhances antioxidant enzyme activities and immunity function in n-methyl-n-nitro-n-nitrosoguanidine-induced gastric cancer rats. *International Journal of Molecular Sciences*, *12*(5), 3340–3351. doi:[10.3390/ijms12053340](https://doi.org/10.3390/ijms12053340)
- Macheix, J. J., Fleuriet, A., & Billot, J. (1990). Fruit phenolics. *CRC Press: Boca Raton, FL, USA*, 101–126.
- Madhuri, B. A., Qairunnisa, S., Suresh, M., Kondam, K. A., & Chandrasekhar, M. (2014). Antioxidant changes in wistar albino rats after acute heat stress. *International Journal of Pharmaceutical Sciences and Research*, *5*(9), 3999.
- Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in burkina fasan honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571–577. doi:[10.1016/j.fochem.2004.10.006](https://doi.org/10.1016/j.fochem.2004.10.006)
- Mukherjee, P. K., Kumar, V., Mal, M., & Houghton, P. J. (2007). Acetylcholinesterase inhibitors from plants. *Phytomedicine*, *14*(4), 289–300.
- Nimse, S. B., & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction

- mechanisms. *RSC Advances*, 5(35), 27986–28006. doi:[10.1039/c4ra13315c](https://doi.org/10.1039/c4ra13315c)
- Oboh, G., & Ademosun, A. O. (2011). Shaddock peels (citrus maxima) phenolic extracts inhibit alpha-amylase, alpha-glucosidase and angiotensin i-converting enzyme activities: A nutraceutical approach to diabetes management. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 5(3), 148–152. doi:[10.1016/j.dsx.2012.02.008](https://doi.org/10.1016/j.dsx.2012.02.008)
- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351–358. doi:[10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- Omoba, O. S., Dada, O. O., & Salawu, S. O. (2015). Antioxidant properties and consumer acceptability of pearl millet - tiger nut biscuits. *Nutrition & Food Science*, 45(6), 818–828. doi:[10.1108/NFS-06-2015-0074](https://doi.org/10.1108/NFS-06-2015-0074)
- Oyaizu, M. (1986). Studies on products of browning reaction. antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44, 307–314. doi:[10.5264/eiyogakuzashi.44.307](https://doi.org/10.5264/eiyogakuzashi.44.307)
- Pellegrino, D. (2016). Antioxidants and cardiovascular risk factors. *Diseases*, 4, 11. doi:[10.3390/diseases4010011](https://doi.org/10.3390/diseases4010011)
- Ranilla, L., Genovese, M., & Lajolo, F. (2007). Polyphenols and antioxidant capacity of seed coat and cotyledon from brazilian and peruvian bean cultivars (phaseolus vulgaris l.) *Journal of Agricultural and Food Chemistry*, 55, 90–8. doi:[10.1021/jf062785j](https://doi.org/10.1021/jf062785j)
- Ross, D. A., Gutierrez-Botero, M., & Van Amburgh, M. E. (2013). Development of an in vitro intestinal digestibility assay for ruminant feeds. 29, 29.
- Saidu, A. N., & Garba, R. (2011). Antioxidant activity and phytochemical screening of five species of capsicum fruits. *International Research Journal of Biochemistry and Bioinformatics*, 1(9), 237–241.
- Salawu, S. O., Alao, O. F., Faloye, O. F., Akindahunsi, A. A., Boligon, A. A., & Athayde, M. L. (2016). Antioxidant potential of phenolic-rich two varieties of nigerian local rice and their anti-cholinesterase activities after in vitro digestion. *Nutrition & Food Science*, 46(2), 171–189. doi:[10.1108/NFS-08-2015-0093](https://doi.org/10.1108/NFS-08-2015-0093)
- Salawu, S. O., Bester, M. J., & Duodu, K. G. (2014). Phenolic composition and bioactive properties of cell wall preparations and whole grains of selected cereals and legumes. *Journal of Food Biochemistry*, 38(1), 62–72. doi:[10.1111/jfbc.12026](https://doi.org/10.1111/jfbc.12026)
- Salawu, S. O., Ajiboye, P. B., Akindahunsi, A. A., & Boligon, A. A. (2017). Antioxidant and anticholinesterase potential of two nigerian bitter yams using a simulated gastrointestinal digestion model and conventional extraction. *Preventive nutrition and food science*, 22(2), 107.
- Sangameswaran, B., Balakrishnan, B. R., Deshraj, C., & Jayakar, B. (2009). In vitro antioxidant activity of roots of thespesia lampas dalz and gibs. *Pakistan Journal of Pharmaceutical Sciences*, 22(4), 368–372.
- Saura-Calixto, F., & Goni, I. (2006). Antioxidant capacity of the spanish mediterranean diet. *Food Chemistry*, 94(3), 442–447. doi:[10.1016/j.foodchem.2004.11.033](https://doi.org/10.1016/j.foodchem.2004.11.033)
- Shahidi, F., & Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. *Journal of Functional Foods*, 5(2), 570–581. doi:[10.1016/j.jff.2013.02.004](https://doi.org/10.1016/j.jff.2013.02.004)
- Shi, J., Dai, Y., Kakuda, Y., Mittal, G., & Xue, S. J. (2008). Effect of heating and exposure to light on the stability of lycopene in tomato puree. *Food Control*, 19(5), 514–520. doi:[10.1016/j.foodcont.2007.06.002](https://doi.org/10.1016/j.foodcont.2007.06.002)
- Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., & Sochor, J. (2015). Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences*, 16(10), 24673–24706. doi:[10.3390/ijms161024673](https://doi.org/10.3390/ijms161024673)
- Szwaĵgier, D. (2014). Anticholinesterase activities of selected polyphenols - a short report. *Polish Journal of Food and Nutrition Sciences*, 64(1), 59–64. doi:[10.2478/v10222-012-0089-x](https://doi.org/10.2478/v10222-012-0089-x)
- Szydłowska-Czerniak, A., Trokowski, K., Karlovits, G., & Szlyk, E. (2011). Effect of refining processes on antioxidant capacity, total contents of phenolics and carotenoids

- in palm oils. *Food Chemistry*, *129*(3), 1187–1192. doi:[10.1016/j.foodchem.2011.05.101](https://doi.org/10.1016/j.foodchem.2011.05.101)
- Tagliazucchi, D., Verzelloni, E., Bertolini, D., & Conte, A. (2010). In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chemistry*, *120*(2), 599–606. doi:[10.1016/j.foodchem.2009.10.030](https://doi.org/10.1016/j.foodchem.2009.10.030)
- Toor, R. K., & Savage, G. P. (2005). Antioxidant activity in different fractions of tomatoes. *Food Research International*, *38*(5), 487–494. doi:[10.1016/j.foodres.2004.10.016](https://doi.org/10.1016/j.foodres.2004.10.016)
- Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*, *7*(1), 65–74. doi:[10.2174/157015909787602823](https://doi.org/10.2174/157015909787602823)
- Vallverdu-Queralt, A., Medina-Rejon, A., Martinez-Huelamo, M., Jauregui, O., Andres-Lacueva, C., & Maria Lamuela-Raventos, R. (2011). Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *Journal of Agricultural and Food Chemistry*, *59*(8), 3994–4001. doi:[10.1021/jf104400g](https://doi.org/10.1021/jf104400g)
- Vinholes, J., Grosso, C., Andrade, P. B., Gil-Izquierdo, A., Valentao, P., de Pinho, P. G., & Ferreres, F. (2011). In vitro studies to assess the antidiabetic, anti-cholinesterase and antioxidant potential of *spergularia rubra*. *Food Chemistry*, *129*(2), 454–462. doi:[10.1016/j.foodchem.2011.04.098](https://doi.org/10.1016/j.foodchem.2011.04.098)
- Violeta, N., Trandafir, I., & Ionica, M. E. (2013). Antioxidant compounds, mineral content and antioxidant activity of several tomato cultivars grown in southwestern romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *41*(1), 136–142.
- Worthington Enzyme Manual. (2009). Worthington biochemical corporation. Retrieved from <http://www.worthington-biochem.com/index/manual.html>
- Yafang, S., Gan, Z., & Jinsong, B. (2011). Total phenolic content and antioxidant capacity of rice grains with extremely small size. *African Journal of Agricultural Research*, *6*(10), 2289–2293.
- Zhang, Y.-J., Gan, R.-Y., Li, S., Zhou, Y., Li, A.-N., Xu, D.-P., & Li, H.-B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, *20*(12), 21138–21156. doi:[10.3390/molecules201219753](https://doi.org/10.3390/molecules201219753)