



Impact of chemical pesticides on antioxidant constituents and free radical scavenging capacity of pesticide-treated tomato (*Solanum lycopersicum* L.) fruits

D.H. Mhya¹, J.S. Muhammad², N.S. Urmar² and A. Mohammed¹

¹Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria
²Department of Biochemistry, Faculty of Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria

ABSTRACT

Tomato fruits are well-known with abundant antioxidants. However, they are often susceptible to pest attacks. To prevent spoilage, tomato fruits are sprayed with pesticides postharvest. On this basis, the study was conducted to examine the impact of pesticide on antioxidant constituents of tomato fruits preserved under pesticides alongside other alternative preservative methods. Tomato fruits grown in a soil-free pesticides were divided into five groups, and each was preserved using different techniques: pesticide spray, freezing, drying, parboiling, and in distilled water which served as a normal control. A mixture of hexane, ethanol, and acetone (2:1:1) was used to extract antioxidant molecules from tomato fruits; the hexane fraction obtained was used to determine the concentration of lycopene, β -carotene, and Vitamins A, C, and E. Antioxidant activities were also investigated. The results of the study revealed that all the methods used in preserving tomato fruit cause alteration in antioxidant molecules, Lycopene in preserved fruits is $\leq 2.52 \pm 0.89$ mg/g FW (fresh weight) compared to 5.07 ± 0.08 mg/g FW (normal control), and β -carotene; $\leq 1.49 \pm 0.09$ mg/g FW compared to 2.99 ± 0.23 mg/g FW (normal control). Vitamin A declined from 30.30 ± 1.79 mg/100g extract (normal control) to $\leq 28.25 \pm 0.49$ mg/100g extract (preserved fruits). Vitamin E in normal control is 5.07 ± 0.08 mg/100g extract, declining to $\leq 4.86 \pm 0.04$ mg/100g extract (preserved fruits). DPPH radical scavenging by fruits preserved under pesticide is 61–70%; other methods is 70–75%; normal control (81.53%); and ascorbic acid (88.50%). In conclusion, preservation of tomato fruits causes a decline in antioxidant molecules, with pesticides causing a greater loss compared to methods like freezing, drying, and parboiling.

Keywords: Antioxidants; Lycopene; β -carotene; Vitamins; Tomatoes; Fruits

INTRODUCTION

It is widely known that fruits are one of the principal sources for the day-to-day intake of healthy constituents in the diet, like minerals, vitamins, and an outspread variety of phytochemicals (Shahidi *et al.*, 2011). Tomato is one of the fruits with a broad spectrum of health benefits. It is rich in antioxidant compounds, and this has placed it as one of the most

important commodities that form a remarkable part of human diets. The bioactive molecules in tomato fruit are carotenoids, particularly lycopene and β -carotene, and vitamins A, C, and E. Lycopene gives the tomatoes a red color; apart from that, it is a powerful antioxidant that helps protect cells from damage by free radicals like hydrogen peroxide, nitrogen dioxide, and hydroxyl radicals (Yin *et al.*, 2019; Imran *et al.*, 2020; Caseiro *et al.*, 2020).

Lycopene has the potential of shielding the eyes from the blue light emitted by gadgets like computers and smartphones. They also help preserve vision and reduce stress associated with eyestrain (Sasaki *et al.*, 2012; Bernstein *et al.*, 2016; Lem *et al.*, 2022). Vitamins A, C, and E are known for their antioxidant potential in the amelioration of oxidative stress; they are reported to have alleviated oxidative stress via free radical scavenging and promoted antioxidant enzymes like superoxide dismutase and glutathione peroxidase activities (Hidayatik *et al.*, 2021).

Beta-carotene is another carotenoid found in tomato fruit. It is a pro-vitamin A carotenoid that produces retinol via retinal cleavage with the β -carotene-15, 15-dioxygenase-1 enzyme (Desmarschelier *et al.*, 2010). Beta-carotene has been known as an important antioxidant (Khachik *et al.*, 1992). It was reported that β -carotene is an excellent singlet oxygen extinguisher and is capable of preventing the formation of singlet oxygen by extinguishing thrilled triplet sensitizers (Demmig-Adams and Adams, 2002).

It is worth noting that the huge potential benefits derive from molecules in tomato fruit have some limitations due to their short shelf life. For this reason, tomato fruits after harvest are subjected to different preserving methods in order to extend their shelf life or prevent their spoilage. Studies reported that most of these methods have varying effects on the bioactive compounds and their activities; this has been a great sort of concern (Mali *et al.*, 2012; Al-Juhaimi *et al.*, 2018). The bioactive compounds in tomato fruits were reported to have been affected by methods applied in trying to preserve them. This is evidenced by a study conducted by Nicoli *et al.* (1999), where processing was found to have caused a liberation or alteration in its molecular content. Dewanto *et al.* (2002) also found that the thermal processing of tomato fruit affects its nutritional value. Their results showed that lycopene concentration increased, attributing to the fact that heating may have promoted its release from its natural matrix.

In addition, processing methods like boiling, microwaving, and frying were found to cause a reduction in quercetin content (82%) lost after tomato fruit was boiled, 65% loss under microwaved processing, and 35% loss when fried (Crozier *et al.* 1997). According to the Krinky and Johnson (2005), boiling tomato fruit does cause changes in carotene concentration. In a recent study, the application of pesticides to tomato seedlings was reported to alter their physiological and biochemical processes, leading to an alteration in the levels of some bioactive molecules (Hatamleh *et al.*, 2022). Findings from the above studies have shown that, alteration of phytoconstituents in tomatoes depends on the method applied in trying to prevent their spoilage. However, whether preserving tomato fruit with chemical pesticides affects its antioxidant constituents remains to be established by research. On this basis, the present study was planned to evaluate the content of antioxidant molecules (lycopene, β -carotene, vitamin A, C, and E) in fresh tomato fruits preserved under pesticides alongside conventional preservative methods.

MATERIALS AND METHODS

Chemicals

All chemicals used are of analytical grade. Pesticides: Z-Force (Mancozeb 80% WP) is a carbamate fungicide use against pest attack affecting field crops, fruits, and vegetables such as tomato.

Plant Sampling/Field Experiment

In the current study, tomato samples were grown in pots at the Botanical Garden of the Abubakar Tafawa Balewa University, Bauchi between October 2022 to March 2023. The soil proportion was 12% clay, 23% silt, and 65% sand—while organic manure was applied as the base fertilizer. Irrigation was given twice in a day. The temperature was in the range of about $30\text{-}35^{\circ}\text{C} \pm 3^{\circ}\text{C}$, with 70–80% humidity all-round the growing period. Fresh tomato fruits were harvested for the study.

Sample Preparation/Preservation

Tomato fruits were thoroughly cleaned to get rid of any dirt by washing with tap water, dried by blotting with a clean cloth, and preserved under different techniques (freezing, drying, par-boiling, and pesticide spray) as follows: Drying: tomatoes were sliced and placed about $\frac{1}{2}$ to 1 inch (1 to 3cm) apart, cut-side up, on a clean wooden tray covered with a fine net to keep insects off in the sun for the duration of 7 days. Freezing: Tomato fruits were placed in a container and frozen at 4°C for 7 days. Par-boiling: Tomato fruits were ground using a blender and concentrated by boiling until semi-solid, then poured into pre-sterilized bottles while still hot. The tomato paste was kept for 7 days before being used for analysis. Pesticide preservation: Fresh tomato fruits were sprayed with different concentrations (1% and 5%) of 50g/15L pesticide (Z-Force®) and kept for 7 days for the pesticides to get absorbed and for possible biochemical reactions. Fresh tomato fruits preserved in distilled water were used as a normal control.

Extraction of Antioxidant Molecules in Preserved Tomato Fruits

Tomato fruits preserved under different techniques were separately pulverized into paste using a pestle and mortar before extraction, while the boiled tomato paste was extracted directly. The extraction process was carried out in the dark to avoid the potential degradation of metabolites, as described by Gomez-Romero *et al.* (2010). Samples (1g) were macerated in a 25-mL mixture of solvents (hexane, ethanol, and acetone in a 2:1:1 volume ratio). The mixture was stirred for 30 minutes at 1500 rpm, and then 10 mL of distilled water was added, and stirring was continued for another 10 minutes. After 15 minutes of rest, the phases were separated. The extraction was carried out in an amber bottle, covered by aluminum foil, in the dark, at room temperature. The obtained hexane extract was then evaporated at 25°C to semi-dryness.

Determination of lycopene and β -carotene contents in Preserved Tomato Fruit Extracts

A method described by Zechmeister and Polgar (1943) was used. Exact 1.0 g of tomato fruit extract was crushed in 2 mL of distilled water in a test tube. The tubes containing the sample were vortexed in a water bath at 30 °C for an hour. The absorbance was then measured at 503 nm and 470 nm. The concentrations of lycopene and β -carotene were determined according to the following formulas:

$$\text{Lycopene conc } \left(\frac{\text{mg}}{\text{g}} \text{ F. W} \right) = [\text{Absorbance of Test}] / [\text{Molar Ext. Coeff of } 172000 \text{ M} - 1 \text{ cm} - 1 \text{ at } 503 \text{ nm}]$$

$$\beta - \text{carotene conc (mg/g F. W)} = [\text{Absorbance of Test}] / [\text{Molar Ext. Coeff. of } 108427 \text{ M} - 1 \text{ cm} - 1 \text{ at } 470 \text{ nm}]$$

Quantification of Antioxidant Vitamins in Preserved Tomato Fruit Extracts

Vitamin A content from tomato fruit was determined using a method described by Rutkowski and Grzegorzczuk (2007). Two (2) mg of extracted tomato fruit was measured in test tubes 1 and 2, and 1M solution of KOH in 90% ethanol was added. The extract was heated (60 °C) for 90 min, cooled, and xylene (2 mL) was added and centrifuged. The supernatant was collected and transferred to test tubes II. The extract was analyzed using 335nm absorbance against xylene before (A1) and after (A2) exposed to UV light for 30 min. The concentration of vitamin A in the extract was determined by applying the formula: Conc. of Vitamin A (mg/100g) = A(1 – A2) * 22.23

Ascorbic acid concentration in tomato fruit was determined using the iodometric method by AOAC (1999). Exact 0.1 g of extracted tomato sample was mixed with a 5 mL solution of meta-phosphoric acid (3%)–acetic acid (7.98%) and centrifuged at 4000 rpm for 10 min, and the supernatant was used for the determination of ascorbic acid. A standard solution of sodium thiosulfate (Na₂S₂O₃) at a concentration of 0.05 mM and a starch indicator were used. A burette was filled with 0.05mM sodium thiosulfate and titrated against the analyte (10 mL of supernatant, 10 mL of iodine solution, and 3 drops of starch indicator) and the standard analyte (10 mL of vitamin C (1%), 10 mL of iodine solution, and 3 drops of starch indicator) until the color changed from blue-black to colorless. The volume of the thiosulfate that resulted in the color change was recorded. To know the concentration of the iodine solution and the amount of ascorbic acid in the sample that reacted with the iodine, the thiosulfate solution was titrated against a blank, which was composed of distilled water, iodine solution, and starch indicator. The concentration of ascorbic acid in the extract was determined by applying the formula:

$$\text{Concentration of Ascorbic acid } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{25y}{b}$$

Where, b is the titer (mL) from the titration of the standard ascorbic acid solution. y = titer (mL) from the titration of the sample solution.

Vitamin E was quantified using the Priero *et al* (1999) method. A 0.1 g of extracted tomato sample was mixed with 10 mL of hexane:isopropanol solution (3:2 v/v), agitated for 5 h and centrifuged at 4000 rpm for 10 min. Exact 0.1 mL of supernatant was mixed in a test tube with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) and incubated at 37°C for 90 min with vigorous shaking. The absorbance of the aqueous phase at 695 nm was measured against the appropriate blank. A

typical blank contained 1 mL of reagent solution and 0.1 mL of pure hexane, and it was incubated under the same conditions as the samples. The quantitation of vitamin E was based on the molar absorption coefficient of the phosphomolybdenum complex using the formula below:

$$\text{Vitamin E} \left(\frac{\text{mg}}{100\text{g}} \text{ extract} \right) = \frac{\text{Absorbance}}{\text{Molar}} \text{Extention Coefficient} (4000\text{M} - 1\text{cm} - 1)$$

DPPH (2, 2-diphenyl-1-picryl hydrazyl) Scavenging Effect of Preserved Tomato Fruit Extracts

The Kedare and Singh (2011) method was used to estimate the antioxidant potential of tomato fruit extract/standard antioxidants by DPPH radical scavenging. The extract/standard antioxidants (0.2–1 mg/mL) was mixed with DPPH solution (6×10^{-5} M solution by combining 2.4 mg of DPPH with 100 mL of ethanol) and incubated at 25°C for 30 minutes. The absorbance was read at 517 nm. The DPPH scavenging ability of each extract was calculated from the decrease in absorbance according to the formula:

$$\text{DPPH Scavenging (\%)} = [\text{Abs of blank} - \text{Abs of Test}] / \text{Abs of blank} * 100$$

Where Abs-blank is the absorbance of the control reaction and Abs-test is the absorbance of the tested sample.

Hydrogen Peroxide Scavenging Effect of Preserved Fruit Extracts

Hydrogen peroxide (H₂O₂) scavenging activity was evaluated according to the method published by Ruch *et al.* (1989). One milliliter of tomato fruit extract ranging from 100 to 500ng/mL was added to 0.6mL of hydrogen peroxide solution (40 mmol) and incubated for 10 minutes at room temperature. The absorbance of the solution at 230 nm was measured in comparison to a blank consisting of phosphate buffer (pH 7.4). The conventional antioxidant (ascorbic acid) was treated in a similar manner. Hydrogen peroxide scavenging activity was estimated using the formula below:

$$\% \text{H}_2\text{O}_2 = [\text{Abs of Control} - \text{Abs of test}] / \text{Abs of Control} * 100$$

Total antioxidant capacity of Preserved Fruit Extracts

The total antioxidant capacity of extracts was assessed based on the reduction of molybdate (VI) to molybdate (V), with the subsequent formation of a green phosphate/MO (V) complex at acid pH as described by Prieto *et al.* (1999). The molybdate reagent solution was made by combining 20 mL of distilled water with 0.1 mL of sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 M). More distilled water was added to the combination until its volume was reduced to 50 milliliters. In a test tube, precisely 10 µL of the extract (1 mg/mL) was combine with 1 mL of the molybdate reagent solution. After 90 minutes of incubation at 95 °C in a water bath, the tune was removed and cooled to room temperature for exactly 30 minutes. Absorbance was read at 695 nm against a blank sample (containing 100 µL of methanol mixed with 900 µL of reagent solution). Ascorbic acid was used as a standard control to plot a curve in which the value of the extract was extrapolated. The antioxidant activity was expressed as mg/g ascorbic acid equivalent (AAE) of dry extract.

Antioxidant Reducing Power of Preserved Fruit Extracts

The Fe³⁺-reducing ability of the extract was identified according to Oyaizu (1986). Extract (0.1 mL) of 200–1000 µg/mL of tomato fruit extract was mixed with 0.25 mL of phosphate buffer (0.2 M, pH 6.6) and 0.25 mL of K₃Fe (CN)₆ (1% w/v). After incubating the mixture at 50 °C in a water bath for 20 min, the reaction was stopped by adding 0.25 mL of trichloroacetic acid solution (10% w/v). Then, the mixture was centrifuged at 5,000 rpm for 10 min. Subsequently, 0.25 mL of the supernatant was mixed with 0.25 mL of distilled water and 0.5 mL of ferric chloride (FeCl₃) solution (0.1% w/v) for 10 min. The absorbance was immediately determined at 700 nm to measure the reducing potential. Ascorbic acid (vitamin C) was used as a reference standard. The increased absorbance of the reaction mixture indicated increased reducing power in the sample.

Statistical Analysis

The data obtained from the study are displayed as means ± standard deviation. One-way analysis of variance (ANOVA) was used for statistical comparisons, and Duncan post hoc analysis was performed using SPSS version 23 software. At $P \leq 0.05$, the significance levels were taken into account.

RESULTS

Extracts Obtained from Extraction of Tomato Fruit

Table 1 displays tomato fruit extract yields according to various methods of preservation. Varied extraction yields were recorded from the tomato fruit preserved following different methods.

Among all the treated fruits, dried tomato fruit yielded the most at 45%, compared to 50% for the unpreserved fruit. The lowest extraction yield at 31% was obtained from tomato fruit treated with pesticides (1%). The investigation noticed several physical alterations during the extraction process, apart from the variation in yields, texture and also color slightly differ. The extracts were semi-solid, greasy, and pale-brownish in color.

Table 1: Extracts obtained from solvent extraction of tomato fruit preserved using conventional and chemical pesticides

	Preserved tomato fruits using conventional and chemical pesticides					
	PTFE	DTFE	FTFE	PTTF1	PTTF5	TFED
Conc. (mg/g Sample)	0.37	0.45	0.33	0.31	0.35	0.50
% Yield	37.0	45.0	33.0	31.0	30.0	50.0

PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PTTF1 = 1% Pesticide; Preserved Tomato Fruit Extract; PTTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

Carotenoid Content of Preserved Fruit Extracts

The observed changes in levels of carotenoid contents in tomato fruits preserved by different methods are presented in Table 2. Lycopene, a major carotenoid in tomatoes, was detected in varied quantities in the range of 1.43±0.26 – 5.07±0.08 mg/g fresh weight (FW),) with the highest value recorded from the normal control (preserved in distilled water) at 5.07±0.08 mg/g FW, followed by the parboiled tomato fruit (4.86±0.04 mg/g FW). The

amount of lycopene in tomato fruit declines as the pesticide concentration increases. Fruit treated with 5% pesticide had a lycopene content of 1.43 ± 0.26 mg/g FW while fruit treated with 1% had 2.52 mg/g FW.

β -carotene was also recorded in extracts obtained from tomato fruits preserved by different techniques between 1.29 ± 0.23 and 2.99 ± 0.23 mg/g FW with the highest value in the normal control (2.99 ± 0.23 mg/g FW). β -carotene value varies intensively; the less was obtained from fruit treated with 5% pesticide concentration at 1.29 ± 0.23 mg/g FW, while the fruit parboiled gave 1.95 ± 0.79 mg/g FW. All the preservation techniques studied seem to affect the composition of β -carotene when compared with the unprocessed one.

Table 2: Variation in the levels of β -arotene and lycopene in fresh tomato fruits preserved using conventional and chemical pesticides

(mg/g FW)	Preserved tomato fruits using conventional and chemical pesticides					
	PTFE	DTFE	FTFE	PTTF1	PTTF5	TFED
β -Carotene	1.84 ± 0.33^d	1.67 ± 0.13^c	1.95 ± 0.79^e	1.49 ± 0.49^b	1.29 ± 0.23^a	2.99 ± 0.23^f
Lycopene	4.86 ± 0.04^e	3.98 ± 0.16^d	3.76 ± 0.03^c	2.52 ± 0.87^b	1.43 ± 0.26^a	5.07 ± 0.08^f

Each value is expressed as mean \pm SD (n=3). Value along the row with different superscript letter is significantly different at $P \leq 0.05$

PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PTTF1 = 1% Pesticide; Preserved Tomato Fruit Extract; PTTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

Antioxidant Vitamins in Preserved Fruit Extracts

Different antioxidant vitamins were determined in the present study, and their results are presented in Table 3. The vitamin concentration varies based on the preservative techniques applied to the tomato fruits, with UTFE having the highest levels of vitamin C (30.30 ± 1.79 mg/100g extract) and vitamin A (2.62 ± 0.08 mg/100g extract), as well as vitamin E (5.07 ± 0.08 mg/100g extract). PTTF5 had the lowest concentrations of vitamin C (25.04 ± 0.46 mg/100g extract), vitamin E (3.67 ± 0.04 mg/100g extract), and vitamin A (1.70 ± 0.06 mg/100g extract), respectively.

Table 3: Variation in content of antioxidant vitamins (a, c, and e) in fresh tomato fruit preserved using conventional and chemical pesticides

Vitamins (mg/100g extract)	Preserved tomato fruits using conventional and chemical pesticides					
	PTFE	DTFE	FTFE	PTTF1	PTTF5	TFED
Vitamin A	2.28 ± 0.16^d	2.16 ± 0.04^c	2.42 ± 0.05^e	2.08 ± 0.03^b	1.70 ± 0.06^a	2.62 ± 0.08^f
Vitamin C	27.14 ± 0.47^b	27.43 ± 0.59^b	28.25 ± 0.49^c	25.33 ± 0.39^a	25.04 ± 0.46^a	30.30 ± 1.79^d
Vitamin E	4.86 ± 0.04^e	3.98 ± 0.16^d	3.76 ± 0.03^b	3.86 ± 0.04^c	3.67 ± 0.04^a	5.07 ± 0.08^f

Each value is expressed as mean \pm SD (n=3). Value along the row with different superscript letter is significantly different ($P \leq 0.05$)
PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PTTF1 = 1% Pesticide; Preserved Tomato Fruit Extract; PTTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

DPPH Scavenging Effect of Preserved Fruit Extracts

Table 4 shows the effects of four preservation methods-freezing, parboiling, drying, and pesticide application on the DPPH scavenging ability of tomato fruit. When extracts obtained from tomato fruit preserved via different methods were studied for their free radical scavenging effect, a decrease in ability was recorded in comparison to standard antioxidant (ascorbic acid; 88.50% and the normal control (81.53%). DPPH radical scavenging for fruits preserved under pesticide is 61–70%; other methods are 70–75%.

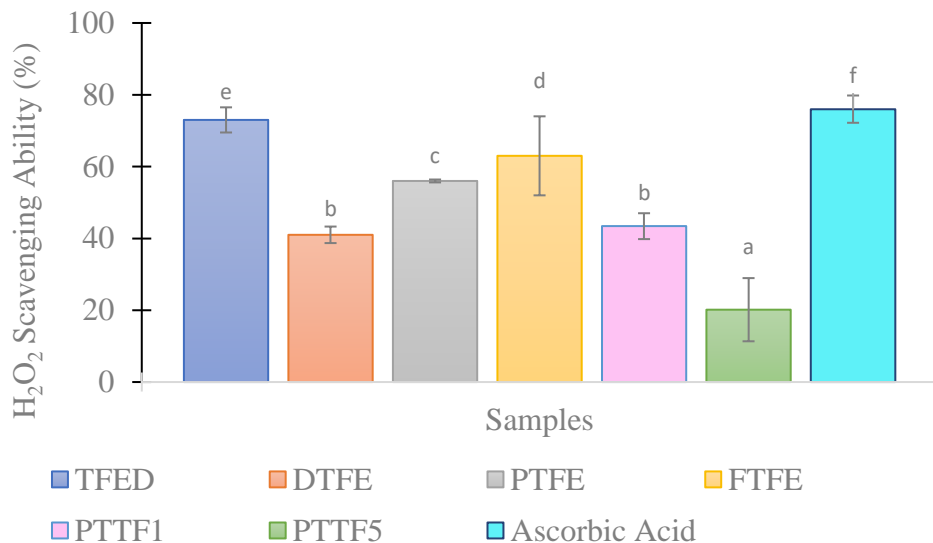
Table 4: DPPH scavenging activity of fresh tomato fruit extracts preserved using conventional and chemical pesticides

Conc. (mg/mL)	Percent (%) DPPH Scavenging Activity of Samples						
	Ascorbic acid	PTPE	DTPE	FTPE	PTTF1	PTTF5	TFED
0.06	55.75	53.31	45.64	35.89	44.25	41.81	49.83
0.12	79.09	56.10	49.13	40.07	50.52	48.43	62.72
0.25	75.96	62.02	53.66	42.51	57.14	53.66	70.38
0.50	87.11	68.99	73.52	72.47	67.52	58.99	77.00
1,00	88.50	73.87	74.56	76.31	70.88	61.08	81.53

PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PTTF1 = 1% Pesticide Preserved Tomato Fruit Extract; PTTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

Hydrogen Peroxide Scavenging Activity of Preserved Tomato Fruit Extracts

The H₂O₂-reducing ability of tomato fruit extract investigated in order to explore its antioxidant potential is presented in Figure 1.



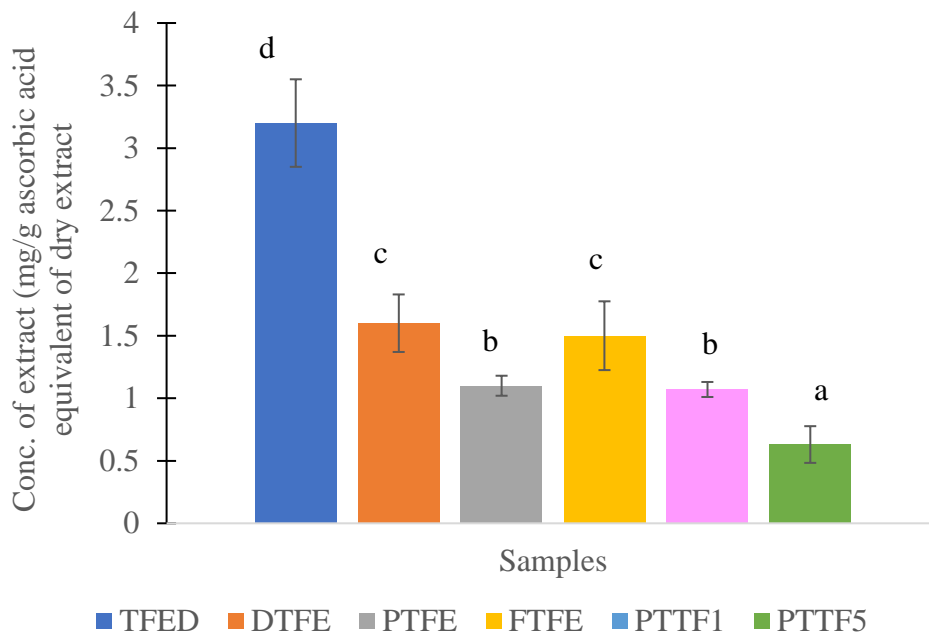
PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PTTF1 = 1% Pesticide Preserved Tomato Fruit Extract; PTTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

Figure 1: Hydrogen peroxide (H₂O₂) scavenging ability of fresh tomato fruit preserved using conventional and chemical pesticides

Results of the analysis showed extracts from tomato fruits subjected to different preservative processes were able to scavenge H₂O₂, but to varying degrees. The free radical scavenging trend of the extracts showed the extract of tomato fruit preserved in distilled had the highest percentage at 73%, followed by the freeze tomato fruit extract at 63%, and the less was from extract of both PTTF5 and PTTF1 at 20.15% and 43.41%.

Total Antioxidant Capacity of Preserved Fruit Extracts

Figure 2 presents the total antioxidant capacity (TAC) of tomato fruit extracts preserved using chemical pesticides in addition to other alternative preservative methods. With a concentration of 1.5mg/g ascorbic acid equivalent to the dry extract, the frozen tomato fruit extract has highest total antioxidant capability among the methods used to preserve tomato fruit. It is followed by the dried and parboiled tomato fruit extracts. The antioxidant capacity of the normal control tomato fruit was found to be much higher (3.2 mg/g ascorbic acid equivalent of dry extract) than that of any of the tomato fruit extracts that were preserved.

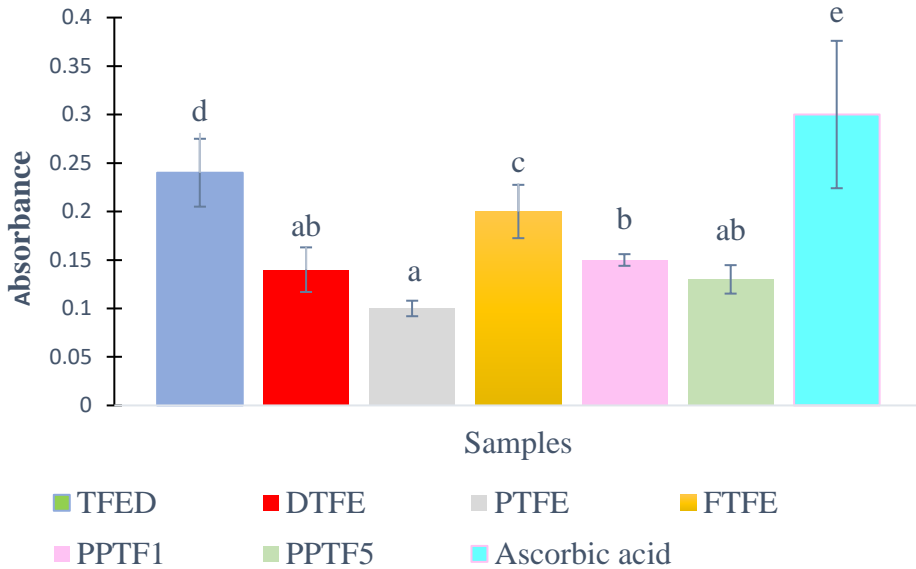


PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PTTF1 = 1% Pesticide Preserved Tomato Fruit Extract; PTTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

Figure 2: The total antioxidant capacity of fresh tomato fruit preserved using conventional and chemical pesticides

Antioxidant Reducing Power of Preserved Fruit Extracts

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. In this study, varied degrees of activity were recorded when extracts from tomato fruit were preserved with chemical pesticides alongside different preservative methods. The standard antioxidant (Vitamin C) had the highest reductive capability at 0.271; others were in the range of 0.05–0.248, with PTFE being the least at 0.1, respectively.



PTFE = Parboiled Tomato Fruit Extract; DTFE = Dried Tomato Fruit Extract; FTPE = Freeze Tomato Fruit Extract; PPTF1 = 1% Pesticide Preserved Tomato Fruit Extract; PPTF5 = 5% Pesticide Preserved Tomato Fruit Extract; TFED (Normal control) = Tomato Fruits Preserved in distilled water

Figure 3: The antioxidant reducing power of fresh tomato fruit preserved using conventional and chemical pesticides

DISCUSSION

This study examined changes in the antioxidant constituents in tomato fruits when preserved under pesticides alongside alternative preservative methods. The study revealed that preserving tomato fruit cause a decline in its phytochemicals, with pesticide application exhibiting the greatest effect. It reduces the amount of chemicals like vitamins and carotenoids, which has an impact on their biological performance. According to scientific studies from the literature, using pesticides on plants can cause changes in the plants' metabolism and as a result, lower the quality of their molecules (Lei *et al.*, 2020; Liu *et al.*, 2021).

It is possible that the present study's findings regarding the reduction in lycopene and β -carotene levels of tomato fruits preserved by freezing, drying, and parboiling processes are related to the fact that different processing techniques have different effects on plant (Mali *et al.*, 2012; Al-Juhaimi *et al.*, 2016). On the other hand, decreased lycopene and β -carotene levels in tomato fruit preserved under chemical pesticide could be that the pesticide might have cause damage to the fruit's cell membrane. According to a study by Hatamleh *et al.* (2022), pesticides cause damage to plant cells, which results in loss of significant amount of molecules. This might explain the variation in the amount of lycopene and β -carotene loss in tomato fruit preserved with a 5% pesticide compared to the 1% pesticide as recorded by the current study suggesting that, at 5% pesticide, a substantial amount evolved into more cell causing more damage.

Activities of enzymatic antioxidant molecules like superoxide dismutase and catalase, among others, were reported to considerably affected by pesticide treatments on plants (Akbuluk *et al.*, 2018; Lian *et al.*, 2020). For instance, superoxide dismutase activity was reported to have been elevated, while catalase activity declined under pesticide-treated plants. The declining activity of catalase was connected to the ability of pesticides to mediate H₂O₂ accumulation. To this effect, poor hydrogen peroxide scavenging noticed by extracts particularly from tomato fruit preserved under pesticide might in part be due to excessive H₂O₂ accumulation within the tomato fruit's cells that might have used up the available catalase in trying to mop it out.

Non-enzymatic antioxidants like vitamin A, ascorbate, and vitamin E have appreciable antioxidant capacity and require impeding the detrimental effects of free radical species. However, the poor free radical scavenging ability demonstrated by extracts from tomato fruits preserved under pesticide may be related to their low levels of non-antioxidant molecules. The decrease in vitamin C concentration recorded in various methods employed for preserving tomato fruit, particularly pesticide application, indicates that pesticides might promote the aging of tomato fruit. According to Cao *et al.* (2023), aging tomato fruit is associated with a decrease in vitamin C levels. In the authors study, it was found that treating fresh tomato fruits with ellagic acid inhibits the decrease in vitamin C levels thereby delaying aging and extending their shelf life.

CONCLUSION

The study found out that tomato fruit's essential components decrease when preserved. Chemical pesticides appear to be more likely to result in the loss of numerous vital components than other traditional preservation techniques. It reduces the amount of chemicals like vitamins and carotenoids, which has an impact on their biological performance. Although using pesticides to preserve tomatoes is possible, it is not recommended due to its devastating effect and health consequence.

Acknowledgements

The authors express their gratitude to the Tertiary Education Trust Fund (TETFUND), Nigeria, for funds support (reference number: TETF/DR&D/CE/UNI/BAUCHI/IBR/2022/V0L.I), the Vice Chancellor (Professor Sani Usman Kunya), the Institutional Base Research (IBR) Directorate (Prof. F.B.J Sawa), and the entire management team of Abubakar Tafawa Balewa University (ATBU), Bauchi, Nigeria, for their approval and for providing the resources needed to carry out the study.

The authors acknowledged Mal. Usman Abubakar of the Department of Medical Biochemistry, Abubakar Tafawa Balewa University Bauchi for his technical assistance.

REFERENCES

- Akbulut, G.B., Yigit, E., Kaya, A., Aktas, A. (2018). Effects of salicylic acid and organic selenium on wheat (*Triticum aestivum* L.) exposed to fenoxaprop-p-ethyl. *Ecotoxicol. Environ. Saf.*, 148, 901–909

- Al-Juhaimi F., Ghafoor, K., Özcan, M.M., Jahurul M.H.A., Babiker, E.E., Jinap, S, Sahena, F., Sharifudin, M.S., & Zaidul, I.S.M. (2018). Effect of various food processing and handling methods on preservation of natural antioxidants in fruits and vegetables. *J Food Sci Technol.* **55**(10), 3872-3880.
- AOAC (1999). Food composition; additives; natural contaminants. Official Methods of Analysis of AOAC, 2. AOAC, Arlington
- Bernstein, P.S., Li, B., Vachali, P.P., Gorusupudi, A., Shyam, R., Henriksen, B.S., & Nolan, J.M (2016). Lutein, zeaxanthin, and meso-zeaxanthin: The basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog. Retin. Eye Res.* **50**, 34–66
- Cao, J.X., Wang, T., Li, Y., Cao, S., & Fan, T.T. (2023). Ellagic acid Treatment Improves Postharvest Quality of Tomato Fruits by Enhancing the Antioxidant Defense System. *J Food Biochem.*, 2023,1-10.
- Caseiro, M., Ascenso, A., Costa, A., Creagh-Flynn, J., Johnson, M., & Simões, S. (2020). Lycopene in human health. *LWT.* **127**,109323.
- Demmig-Adams, B., & Adams, W.W. (2002). Antioxidants in photosynthesis and human nutrition. *Science*, **298**, 2149–53
- Desmarchelier, C., Landrier, J.F., & Borel, P. (2018). Genetic factors involved in the bioavailability of tomato carotenoids. *Current Opinion in Clinical Nutrition and Metabolic Care*, **21** (6), 489-497.
- Dewanto, V., Wu, X., Adom, K.K., & Liu, R.H. (2002). Thermal processing enhances the nutritional value of Tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.*, **50**,3010–3014.
- Gomez-Romero, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry.* **71**,1848–1864.
- Hatamleh, A.A., Danish, M., Al-Dosary, M.A., El-Zaidy, M., & Ali, S. (2022). Physiological and oxidative stress responses of *Solanum lycopersicum* (L.) (tomato) when exposed to different chemical pesticides. *RSC Adv.*, **12**(12),7237-7252.
- Hidayatik, N., Purnomo, A., Fikri, F., & Purnama, M.T.E. (2021). Amelioration on oxidative stress, testosterone, and cortisol levels after administration of Vitamins C and E in albino rats with chronic variable stress. *Vet World*, **14**(1),137-143.
- Imran, M., Ghorat, F., Ul-Haq, I., Ur-Rehman, H., Aslam, F., Heydari, M., Shariati, M.A., Okuskhanova, E., Yessimbekov, Z., Thiruvengadam, M., Hashempur, M.H., & Rebezov, M. (2020). Lycopene as a Natural Antioxidant Used to Prevent Human Health Disorders. *Antioxidants (Basel)*, **9**(8),706.
- Kedare, S.B., & Singh, R.P. (2011). Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol.*, **48**(4), 412-22.
- Khachik, F., Goli, M.B., Beecher, G.R., Holden, J., Lusby, W.R., Tenorio, M.D., & Barrera, M.R (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J. Agric. Food Chem.*, **40**, 390–398.
- Krinsky, N.I., & Johnson, E.J., (2005). Carotenoid actions and their relation to health and disease. *Mol Aspects Med.* **26**,459–516
- Lei, M., Raza, I., Deebea, F., Jamil, M., Naeem, R., Azizullah, A., Khattak, B., Shah, A., Ali, Z.S., & Khan, M.D. (2020). Pesticide-Induced Physiological, Metabolic and

- Ultramorphological Alterations in Leaves of Young Maize Seedlings. *Pol. J. Environ. Stud.*, 29(3), 2247-2258.
- Lem, D.W., Gierhart, D.L., & Davey, P.G. (2022). Can Nutrition Play a Role in Ameliorating Digital Eye Strain? *Nutrients*, 14(19), 4005.
- Lian, J., Zhao L., Wu, J., Xiong, H., Bao, Y, Zeb, A, Tang, J., & Liu, W. (2020). Foliar spray of TiO₂ nanoparticles prevails over root application in reducing Cd accumulation and mitigating Cd-induced phytotoxicity in maize (*Zea mays* L.). *Chemosphere*, 239, 124794.
- Liu, T., Li, T., Zhang, L., Li, H., Liu, S., Yang, S., An, Q., Pan, C., & Zou, N. (2021). Exogenous salicylic acid alleviates the accumulation of pesticides and mitigates pesticide-induced oxidative stress in cucumber plants (*Cucumis sativus* L.). *Ecotoxicology and Environmental Safety*, 208, 111654.
- Mali, A., Khedkar, K., & Lele, S. (2012). Effect of gamma irradiation on total phenolic content and in vitro antioxidant activity of pomegranate (*Punica granatum* L.) peels. *Food Nutr Sci.*, 2, 428-433.
- Nicoli, M., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends Food Sci. Technol.*, 10, 94–100.
- Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *The Japanese Journal of Nutrition and Dietetics*, 44, 307–315.
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*, 269, 337-41.
- Ruch, R.J., Cheng, S.J., & Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10, 1003–1008.
- Rutkowski, M., & Grzegorzczak, K. (2007). Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. *Acta Scientiarum Polonorum: Technologia Alimentaria*, 6(3),17–28.
- Sasaki, M., Yuki, K., & Kurihara, T. (2012). Biological role of lutein in the light-induced retinal degeneration. *The Journal of Nutritional Biochemistry*, 23 (5), 423-429
- Yin, Y., Zheng, Z., & Jiang, Z. (2019). Effects of lycopene on metabolism of glycolipid in type 2 diabetic rats. *Biomed. Pharmacother.*, 109, 2070–2077.
- Zechmeister, L., & Polgar, A. (1948). Cis-trans Isomerization and spectral characteristics of carotenoids and some related compounds. *Journal of the American Chemical Society*, 65(8),1522-1528.