

Impact of various preservation methods on the antioxidant profile and stress biomarkers of tomato (*Solanum Lycopersicum*)

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Abstract

Tomatoes (*Solanum lycopersicum*) are vital sources of dietary antioxidants, but their post-harvest shelf-life is limited by rapid physiological degradation. Identifying preservation techniques that maintain the biochemical integrity of the fruit is essential for reducing food waste and retaining nutritional value. This study evaluated the impact of five preservation techniques; freezing, shade drying, microwave drying, hot air drying, and sun drying on the antioxidant capacity, glutathione homeostasis, and enzymatic defense systems of tomato fruit. Fresh tomatoes (control) and preserved samples were analyzed for total antioxidant capacity (TAC), lipid peroxidation markers; malondialdehyde (MDA) and 4-hydroxynonenal (4-HNN), reduced and oxidized glutathione (GSH and GSSG), levels and the activity of key antioxidant enzymes, including Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), and Glutathione Peroxidase (GP). Preservation led to a progressive decline in antioxidant capacity, with IC₅₀ values increasing from 13.49 µg/mL in fresh samples to 25.81 µg/mL in sun-dried samples. Freezing was the most effective method, maintaining GSH levels (63.52 µg/g) and oxidative stress markers MDA and 4-HNN (2.14 and 1.02 µg/g) closest to fresh control values ($p > 0.05$). Conversely, thermal dehydration methods particularly sun drying, induced significant oxidative stress ($p < 0.05$), evidenced by a 95% increase in MDA (4.02 µg/g) and a sharp rise in 4-HNN and GSSG. The GSH/GSSG ratio, a critical indicator of cellular redox status, plummeted from 8.95 in fresh samples to 2.27 in sun-dried samples. Furthermore, enzymatic activities (CAT, SOD, GR, and GP) showed significant depletion across all drying methods, with sun drying exhibiting the lowest residual activity. Our findings showed that freezing is superior for preserving the biochemical and antioxidant integrity of tomatoes. While drying methods extend shelf-life, they significantly compromise the enzymatic defense system and increase lipid peroxidation, with sun drying being the most detrimental to the fruit's nutritional quality.

Keywords: *Solanum lycopersicum*; Glutathione; Lipid Peroxidation; Antioxidant Enzymes; Food Preservation; IC₅₀

1. Introduction

Tomato is one of the most widely consumed horticultural crops globally, serving as a fundamental component of the human diet [1]. Renowned for its rich nutritional profile, it provides essential vitamins, minerals, and a potent array of bioactive compounds, including lycopene, phenolics, and a sophisticated internal antioxidant defense system [2]. However, tomato is a highly perishable climacteric fruit, characterized by a high moisture content and a rapid respiration rate, which makes it susceptible to significant post-harvest qualitative and quantitative losses. At the cellular

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level, the senescence and post-harvest degradation of tomatoes are closely linked to the overproduction of reactive oxygen species (ROS) [3]. To mitigate the damaging effects of ROS such as lipid peroxidation and protein denaturation, tomato utilizes a complex antioxidant network [4]. Central to this network is Glutathione, a non-enzymatic antioxidant that plays a pivotal role in the ascorbate-glutathione cycle [5]. In pro-oxidant conditions, two GSH molecules dimerize through a disulfide bond to form GSSG, whereas in antioxidant conditions GSSG is converted to GSH via NADPH-dependent GR enzyme [6]. The GSH/GSSG ratio serves as a vital biomarker for cellular redox status; a decline in this ratio is linked to the pathogenesis of neurodegenerative and neuropsychiatric conditions, including Alzheimer's and Parkinson's diseases [7]. As a potent endogenous antioxidant, GSH is essential for neutralizing harmful reactive oxygen and nitrogen species across all tissues. Within the brain, depleted GSH levels compromise antioxidant defenses, leading to the accumulation of ROS and subsequent oxidative stress [8]. GSH act as a primary defense against oxidative stress by maintaining the cellular redox state and serving as a substrate for various protective enzymes [9]. The efficiency of this defense system is further supported by key antioxidant enzymes, including SOD, CAT, and GP. SOD acts as the first line of defense by disproportionating superoxide radicals into hydrogen peroxide (H_2O_2), which is subsequently detoxified by CAT and GP [10]. The synergy between these enzymes and the TAC determines the fruit's ability to withstand environmental stressors during storage.

Despite the importance of these biochemical markers, common preservation methods ranging from low-temperature storage to various dehydration techniques like sun and microwave drying, exert different degrees of thermal and physical stress on the fruit's tissue [11][12][13]. While these methods are intended to extend shelf-life, they often trigger a breakdown in the redox balance, leading to the accumulation of oxidative stress biomarkers such as MDA and 4-HNN [14]. MDA is the final byproduct of lipid peroxidation triggered by free radicals [15]. 4-HNN is a highly reactive lipid peroxidation product derived from the oxidation of omega-6 polyunsaturated fatty acids. Due to its electrophilic nature, 4-HNN readily forms covalent adducts with proteins, DNA, and lipids, leading to significant cellular dysfunction and impaired molecular signaling [16].

The nutritional profile of dried fruits and vegetables is heavily dictated by preservation parameters. Several studies have indicated that, factors such as thermal intensity, duration of exposure, and light levels play a critical role in determining the final vitamin concentration and overall nutrient retention [17][18]. As a significant source of antioxidant vitamins and bioactive compounds, tomatoes are integral to a health-conscious diet [19]. Nevertheless, post-harvest preservation and processing can drastically alter their biochemical profile, specifically affecting the stability of antioxidant systems [20]. Because tomatoes spoil quickly, using the right preservation method is essential for keeping them fresh and nutritious. While drying is a popular way to extend their shelf life, the specific technique used, whether it's sun-drying, oven-drying, or freeze-drying, significantly impacts the final product's quality, nutrient levels, and antioxidant power [13][21].

Given the rising consumer demand for processed tomato derivatives, evaluating how various preservation methods influence nutritional integrity is critical. This study systematically examines the impact of freezing and diverse dehydration techniques including shade, sun, hot-air, and microwave drying on glutathione homeostasis (GSH/GSSG), oxidative stress biomarkers (MDA and 4-HNN), activity of key antioxidant enzymes (CAT, SOD, GR, and GP) and TAC in tomato. By quantifying the shift in the GSH/GSSG ratio and the resulting changes in enzymes activity and IC_{50} values, this research seeks to identify the most effective preservation strategy for maintaining the functional and nutritional integrity of this vital crop.

2. Materials and methods

2.1. Materials

Fresh tomatoes were harvested from a farm in Kabo Local Government Area of Kano State, Northern Nigeria. The collected samples were cleaned immediately. Botanical identification was performed at the Department of Biological Sciences, Bayero University Kano. For the experimental setup, three samples were randomly taken into each container. While fresh samples were analyzed immediately upon collection, the shade-dried, sun-dried, hot-air-dried, and microwave-dried samples were analyzed after a ten-day drying period. The frozen samples were stored at $-20^{\circ}C$ until required for assay.

2.2. Drying process

2.2.1. Shade drying

Fresh samples were shade-dried in a well-ventilated indoor environment under indirect sunlight for nine days, achieving a total weight reduction of 75% [13].

2.2.2. Hot air drying

To prepare the dried samples, the tomatoes were sliced to a 3 mm thickness and placed in a dryer at 50°C for 24 hours. The target residual moisture content was 15–20%. Throughout the process, temperature was regulated by a thermostat and cross-checked with a laboratory thermometer. Fresh samples were homogenized for three minutes using a hand blender before undergoing analysis based on the method of Ibrahim et al. [13] and Mendelová et al. [22].

2.2.3. Microwave drying

Fresh tomato samples were weighed and subjected to microwave irradiation at full power for six cycles of five minutes each. Following this treatment, a total weight loss of 75% was achieved, in accordance with the method described by Baker et al. [11] and Ibrahim et al. [13].

2.2.4. Sun drying

In accordance with Baker et al. [11] and Ibrahim et al. [13], samples were sun-dried outdoors under well-ventilated conditions for seven days to a total weight loss of 75%.

2.3. Determination of Glutathione and MDA

Homogenized tomato samples (2.0 g) were treated with 1.00 mL of 0.50 M perchloric acid to precipitate proteins, followed by a 10-minute incubation in a sonicator. The mixture was diluted to a final volume of 6.0 mL with distilled water, vortexed, and centrifuged at 6000 rpm for 5 minutes. The resulting supernatant was filtered using Whatman No. 1 filter paper and transferred to 1.0 mL HPLC vials. Concentrations of reduced and oxidized glutathione, as well as MDA, were determined following the methodology of Ibrahim et al. [23]. HPLC analysis was performed using an Exsil 100-5 ODS column (25 cm × 4.6 mm ID, 5 µm) with a mobile phase consisting of 50 mM NaClO₄ in 1% H₃PO₄ (pH 4.0) at a flow rate of 1.0 mL/min. Detection wavelengths were set at 215 nm for glutathione and 254 nm for MDA.

2.4. Determination of 4-HNN

Homogenized tomato samples (2.0 g) were collected in tubes. To each tube, 6.0 mL of ethanol was added, followed by vortexing and ultrasonic treatment for 7.5 minutes to facilitate cell disruption. After sonication, the samples were taken into three separate tubes (2 mL each), and 1.0 mL of n-hexane was added to each before centrifuging for 6 minutes. The n-hexane phase was extracted, and this process was repeated twice. The pooled n-hexane fractions were transferred to glass tubes and evaporated under vacuum. The resulting residue was reconstituted in 1.0 mL of methanol via vortexing. Finally, 1.0 mL of the sample was transferred to HPLC vials for analysis. Chromatographic separation was performed using an ODS-2 column (25 cm × 4.6 mm ID, 5 µm) with a mobile phase of methanol, acetonitrile, and water (33:63:4 v/v) at a flow rate of 1.0 mL/min. The 4-HNN peaks were detected at a wavelength of 232 nm [11][23].

2.5. Enzymes activity determination

kits from Solarbio Life Science were utilized for SOD (BC0170), CAT (BC0200), GR (BC1170), and GP (BC1190). 150 µL of each sample and 1 mL of the extraction reagent were combined and properly mixed in an ice bath in accordance with the manufacturer's instructions. The supernatant was obtained by centrifuging the mixture for 10 minutes at 4 °C and 8000 rpm. After precisely 90 µL of the mixture was collected using a glass cuvette, it was carefully combined with 180 µL of reagent III, 240 µL of reagent I, 6 µL of reagent II, and 30 µL of reagent V. The mixture was then mixed with 180 µL of distilled water and let to stand at 4°C for half an hour. The mixtures of each sample were tested for the activity of SOD, CAT, GR and GP using Spectrophotometer, as also used by Nafiu et al. [24] and Ibrahim et al. [25].

2.6. Total antioxidant capacity determination

The antioxidant capacity was measured according to the method based on the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described by Cakmak et al. [14] and Nile et al. [26] and A solution of 25 µg/mL DPPH in methyl alcohol was prepared, and the absorption of DPPH solution was measured at 510 nm. Then different amount of the sample extracts was added to DPPH solution and kept in dark for 30 minutes before

measurement of absorbance at 510 nm. The percentage of DPPH scavenging effect was calculated using the following equation (1):

$$\text{DPPH scavenging effects (\%)} \text{ or percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 is the absorbance of the sample free DPPH solution, and A_1 is the absorbance of the sample containing DPPH solution after 30 minutes. The results of DPPH were given as IC_{50} ($\mu\text{g/mL}$). The IC_{50} values indicates the concentration of the antioxidant substance which inhibits 50% of the DPPH radical in the medium. Low IC_{50} values indicate high antioxidant activity.

2.7. Statistical analysis

All experimental measurements were performed in triplicate, with results expressed as the mean $\pm$ standard deviation. Data were subjected to analysis of variance (ANOVA) using SPSS version 10.0 for Windows. Statistical significance was defined at $p < 0.05$.

3. Result

Glutathione is an important antioxidant that neutralizes free radicals [27]. The level of GSH in this study was found to be 64.12 ± 1.77 $\mu\text{g/g}$. After application of different preservation methods, the values were found to be 63.52 ± 1.55 , 49.34 ± 2.01 , 43.73 ± 1.12 , 38.56 ± 2.61 and 29.86 ± 1.12 $\mu\text{g/g}$ in frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 1), where a significant decrease ($p < 0.05$) was seen in all the values when compared with the value of the fresh sample (control) except that of the frozen sample.

The level of GSSG was found to be 7.16 ± 0.46 $\mu\text{g/g}$. After application of different preservation methods, the values were found to be 7.43 ± 1.02 , 8.19 ± 1.65 , 10.76 ± 1.56 , 11.87 ± 2.32 and 13.14 ± 1.31 $\mu\text{g/g}$ in frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 1), where a significant increase ($p < 0.05$) was seen in all the values when compared with the value of the fresh sample (control).

The ratio of GSH/GSSG was found to be 8.95 in the control. After various treatment, the values decreased to 8.54, 6.02, 4.06, 3.24 and 2.27 in frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 1).

MDA and 4-HNN are products of lipid peroxidation [28]. In this work, the content of MDA was observed to be 2.06 ± 0.06 $\mu\text{g/g}$. After application of different preservation methods, the values were found to be 2.14 ± 0.08 , 2.85 ± 0.11 , 3.12 ± 0.17 , 3.78 ± 0.04 and 4.02 ± 0.18 $\mu\text{g/g}$ in frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 1), where a significant increase ($p < 0.05$) was seen in all the values when compared with the value of the fresh sample (control) except that of the frozen sample.

In this study also, the content of 4-HNN was seen to be 0.97 ± 0.05 $\mu\text{g/g}$. After application of different preservation methods, the values were found to be 1.02 ± 0.06 , 1.13 ± 0.03 , 1.76 ± 0.09 , 1.96 ± 0.08 and 2.19 ± 0.11 $\mu\text{g/g}$ in frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 1), where a significant increase ($p < 0.05$) was seen in all the values when compared with the value of the fresh sample (control).

Table 1 The contents of MDA, 4-HNN, GSH and GSSG in fresh, frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato ($\mu\text{g/g}$)

	Fresh	Frozen	Shade dried	Microwave dried	Hot air dried	Sun dried
MDA	2.06 ± 0.06	2.14 ± 0.08	2.85 ± 0.11	3.12 ± 0.17	3.78 ± 0.04	4.02 ± 0.18
4-HNN	0.97 ± 0.05	1.02 ± 0.06	1.13 ± 0.03	1.76 ± 0.09	1.96 ± 0.08	2.19 ± 0.11
GSH	64.12 ± 1.77	63.52 ± 1.55	49.34 ± 2.01	43.73 ± 1.12	38.56 ± 2.61	29.86 ± 1.12
GSSG	7.16 ± 0.46	7.43 ± 1.02	8.19 ± 1.65	10.76 ± 1.56	11.87 ± 2.32	13.14 ± 1.31
GSH/GSSG	8.95	8.54	6.02	4.06	3.24	2.27

Antioxidant enzymes (SOD, CAT, GP, and GR) are the body's built-in defense system. They combat oxidative stress by transforming unstable free radicals into safe, stable molecules, preventing cellular damage at the source [29]. In the present work, the activity of CAT was found to be 7.21 ± 0.09 U/mg protein. However, the activity significantly goes down ($p < 0.05$) to 6.98 ± 1.03 , 6.47 ± 0.87 , 4.55 ± 0.14 and 3.82 ± 0.07 U/mg protein in shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 2). Similarly, the activity of SOD was found to be 54.63 ± 3.97 U/mg protein. However, the activity significantly goes down ($p < 0.05$) to 47.38 ± 5.71 , 32.22 ± 2.77 , 28.97 ± 2.33 and 24.76 ± 3.96 U/mg protein in shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 2). In the present work, the activity of GR was found to be 89.84 ± 7.31 U/mg protein. However, the activity significantly decreased ($p < 0.05$) to 63.61 ± 4.35 , 59.76 ± 4.39 , 51.04 ± 6.91 and 47.33 ± 2.51 U/mg protein in shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 2). The present study shows the activity of GP to be 110.53 ± 6.72 U/mg protein which significantly reduced to 91.32 ± 6.22 , 72.26 ± 9.83 , 64.12 ± 8.33 and 49.84 ± 5.32 U/mg protein in shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 2).

Table 2 The activities of CAT, SOD, GR and GP in fresh, frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato (U/mg protein)

	Fresh	Frozen	Shade-dried	Microwave dried	Hot air dried	Sun dried
CAT	7.21 ± 0.09	7.11 ± 0.13	6.98 ± 1.03	6.47 ± 0.87	4.55 ± 0.14	3.82 ± 0.07
SOD	54.63 ± 3.97	54.42 ± 6.21	47.38 ± 5.71	32.22 ± 2.77	28.97 ± 2.33	24.76 ± 3.96
GR	89.84 ± 7.31	85.12 ± 5.51	63.61 ± 4.35	59.76 ± 4.39	51.04 ± 6.91	47.33 ± 2.51
GP	110.53 ± 6.72	107.62 ± 9.81	91.32 ± 6.22	72.26 ± 9.83	64.12 ± 8.33	49.84 ± 5.32

The IC_{50} is a measure of antioxidant capacity which refers to the concentration of the antioxidant substance that inhibits 50% of the DPPH radical in the medium. The antioxidant capacity is inversely proportional to IC_{50} value [30]. The IC_{50} recorded from our work in the fresh tomato sample was found to be $13.49 \mu\text{g/mL}$. The lower the IC_{50} , the higher the antioxidant capacity. After different treatment, the value was found to be 14.57, 18.42, 20.51, 22.66 and $25.81 \mu\text{g/mL}$ in frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato respectively (Table 3).

Table 3 The total antioxidant capacity in fresh, frozen, shade dried, microwave dried, hot air dried and sun-dried samples of tomato

	Fresh	Frozen	Shade dried	Microwave dried	Hot air dried	Sun dried
IC_{50} ($\mu\text{g/mL}$)	13.49	14.57	18.42	20.51	22.66	25.81

4. Discussion

4.1. Effect of different preservation techniques on glutathione

Glutathione, a tripeptide essential for neutralizing deleterious reactive oxygen and nitrogen species, demonstrated extreme sensitivity to thermal and oxidative processing [31]. In our fresh samples, GSH levels were recorded at $64.12 \pm 1.77 \mu\text{g/g}$. However, we observed a progressive decline in GSH content across drying methods, culminating in a 53.4% loss in sun-dried samples ($29.86 \pm 1.12 \mu\text{g/g}$). As noted by De Melo-Marins et al. [32], high temperatures facilitate the cleavage of chemical bonds between the constituent amino acids (cysteine, glycine, and glutamate) by effectively inactivating the molecule. Furthermore, the significant increase in GSSG from $7.16 \pm 0.46 \mu\text{g/g}$ in fresh samples to $13.14 \pm 1.31 \mu\text{g/g}$ in sun-dried samples indicates that the drying process promotes the oxidation of GSH. This is further evidenced by the sharp decline in the GSH/GSSG ratio from 8.95 to 2.27. Such a reduction in the redox ratio suggests that the rate of GSH oxidation exceeds the tissue's capacity for enzymatic regeneration, a phenomenon also observed by Cakmak et al. [14] in *Opuntia ficus-indica* fruit. Saito [33] confirms that the combination of sustained heat and atmospheric oxygen exposure during drying is the primary driver of this antioxidant depletion in tomatoes.

4.2. Effect of different preservation techniques on MDA and 4-HNN

The depletion of the glutathione shield appears to leave the cellular lipid membranes vulnerable to attack, as evidenced by the rise in lipid peroxidation markers. MDA, a byproduct of the breakdown of polyunsaturated fatty acids, rose significantly ($p < 0.05$) from $2.06 \pm 0.06 \mu\text{g/g}$ in fresh samples to $4.02 \pm 0.18 \mu\text{g/g}$ in sun-dried samples. This doubling of

MDA levels indicates a state of severe oxidative imbalance [15]. Parallel to the rise in MDA, the content of 4-HNN often termed a "toxic second messenger" increased from $0.97 \pm 0.05 \mu\text{g/g}$ to $2.19 \pm 0.11 \mu\text{g/g}$. The accumulation of 4-HNN is particularly damaging because it forms covalent adducts with DNA and proteins, which directly impairs mitochondrial function and creates a self-sustaining cycle of cellular injury [34]. The fact that freezing did not significantly alter MDA levels compared to the control suggests that low-temperature preservation successfully inhibits the chain reactions of lipid peroxidation that are otherwise accelerated by heat.

4.3. Effect of different preservation techniques on enzymes activity

The results of the present study demonstrate a significant reduction ($p < 0.05$) in the activity of key enzymatic antioxidants; CAT, SOD, GR, and GP across all drying treatments compared to fresh tomato samples. These enzymes constitute the primary line of defense against ROS, and their decline serves as a critical indicator of thermal and oxidative degradation during processing [35][36]. A consistent trend was observed across all four enzymes, where the degree of activity loss followed the order: Fresh < Shade Dried < Microwave Dried < Hot Air Dried < Sun-Dried. Shade drying exhibited the highest retention of enzymatic activity. As noted by Chiozzi, et al. [37], the absence of direct UV radiation and lower ambient temperatures preserve the structural integrity of the protein globule.

Conversely, sun-drying resulted in the most drastic reductions. The synergistic effect of prolonged heat and solar UV radiation has been shown to accelerate the cleavage of peptide bonds and the oxidation of amino acid side chains, particularly in sensitive enzymes like CAT [38][39]. The significant drop in the activities of CAT (7.21 to 3.82 U/mg protein) and SOD (54.63 to 24.76 U/mg protein) highlights the thermal instability of these metalloenzymes. SOD is responsible for the dismutation of superoxide radicals into H_2O_2 , which is subsequently neutralized by CAT [40]. Research suggests that thermal processing at temperatures exceeding 50°C disrupts the metal-coordinated active sites such as the heme group in CAT or the Cu/Zn sites in SOD, rendering the enzymes catalytically inactive [41][42][43].

The activities of GR and GP were equally compromised. The decline in GP activity (from 110.53 in fresh sample to 49.84 U/mg protein in sun-dried samples) is particularly critical, as GP is essential for reducing hydroperoxides using GSH as a cofactor [44]. The concurrent reduction in GR, the enzyme responsible for regenerating reduced GSH from its oxidized form GSSG [45] suggests a collapse of the glutathione-ascorbate cycle [46][47]. As GR activity diminishes, the cell's ability to maintain a high GSH/GSSG ratio is lost, leading to the accumulation of ROS and the subsequent formation of toxic byproducts like MDA and 4-HNN.

While microwave drying was more destructive than shade drying, it preserved significantly more enzymatic activity than hot air drying. This is likely due to the shorter processing time required. According to Cao et al. [48], the rapid removal of moisture during microwave heating "locks" enzymes into a dehydrated, stable glass-like state before prolonged thermal exposure can cause full denaturation. In contrast, the continuous flow of oxygenated air in conventional ovens ($60\text{--}70^\circ\text{C}$) facilitates sustained oxidative damage to the enzyme's protein backbone.

4.4. Effect of different preservation techniques on total antioxidant capacity and IC_{50} Values

To quantify the cumulative effect of these biochemical changes, we utilized the DPPH radical scavenging assay. The IC_{50} value, which is inversely proportional to antioxidant capacity [30], increased across all treatments. The fresh tomato samples exhibited the lowest IC_{50} ($13.49 \mu\text{g/mL}$), representing the highest potency. In contrast, the sun-dried samples required nearly double the concentration ($25.81 \mu\text{g/mL}$) to achieve the same 50% inhibition. These findings are consistent with the reduced Trolox Equivalent Antioxidant Capacity (TEAC) observed in our previous studies [13], reinforcing the conclusion that drying methods, particularly sun and hot-air drying drastically diminish the total antioxidant potential. The data suggest that the loss of potency is a direct result of the simultaneous degradation of endogenous antioxidants like GSH and the emergence of pro-oxidative byproducts like MDA and 4-HNN.

4.5. Comparison of preservation techniques

Among the methods tested, freezing was the only technique that did not result in a significant decrease in enzymes activity, GSH content or an increase in MDA level ($p > 0.05$), making it the superior method for nutrient retention. Among the drying methods, shade drying was the most effective at preserving the GSH/GSSG ratio and minimizing the IC_{50} , likely due to the absence of direct UV radiation and lower thermal stress compared to microwave and sun-drying.

5. Conclusion

The present study provides a comprehensive biochemical evaluation of how various preservation methods; freezing and four distinct drying techniques alter the redox homeostasis and antioxidant architecture of tomatoes. Our findings

reveal that the preservation of the "master antioxidant," glutathione, is inextricably linked to the stability of enzymatic defense systems and the suppression of lipid peroxidation. The data demonstrates a clear hierarchy in preservation efficacy. Freezing emerged as the most effective method, maintaining GSH levels and enzymatic activities (CAT, SOD, GR, and GP) nearest to fresh control values, while effectively inhibiting the formation of the toxic lipid peroxidation markers MDA and 4-HNN. Conversely, traditional sun-drying and hot-air drying induced the most severe oxidative damage. The catastrophic collapse of the GSH/GSSG ratio in these samples indicates a failure of the glutathione-ascorbate cycle, which directly facilitated the accumulation of reactive aldehydes and a subsequent doubling of MDA and 4-HNN concentrations.

Furthermore, the total antioxidant capacity, quantified through IC₅₀ values, showed a significant inverse correlation with the degree of thermal and oxidative stress. The sharp increase in IC₅₀ across drying treatments, particularly in sun-dried samples confirms a profound loss of radical-scavenging potency. Among the dehydration methods, shade drying and microwave drying offered superior bioactive retention compared to conventional methods, likely due to reduced UV exposure and shorter processing durations, respectively.

While dehydration remains essential for shelf-life extension, it carries a significant "enzymatic and antioxidant cost." These results suggest that for the production of functional tomato-based products, low-temperature freezing or optimized shade/microwave drying should be prioritized to mitigate oxidative injury. This work underscores the necessity of monitoring reactive aldehydes like 4-HNN alongside traditional antioxidants to fully assess the nutritional safety and quality of processed vegetables. Furthermore, these findings have implications for the pharmaceutical and food industries, where preserving bioactive compounds is crucial. Further research on optimizing preservation techniques could enhance the nutritional and medicinal value of tomato-based products.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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