**Influence of processing and storage on the health benefits of sauce produced from two *Capsicum* spp (Pepper) and *Lycopersicum esculentum* (tomato): In CCl4 induced hepatotoxic wistar rats**

**Abstract**

In nutraceutics, a wide range of nutritious foods are being explored for their pharmacological benefits. Pepper and tomato fruits which can be processed into different products like sauce, soups, paste, and other delicacies are packed with health-promoting substances. Carbon tetrachloride (CCl4) has been described as an environmental toxicant known to affect human health and to induce hepatotoxicity in experimental animals. This study investigated the influence of processing with heat treatment and storage on the biological role of sauce produced from pepper (*Capsicum annum*, *Capsicum chinense*) and tomato (*Lycopersicum esculentum*). This was evaluated in rats induced with hepatotoxicity using CCl4 and fed a diet supplemented with the sauce. They were administered a single intraperitoneal dose of CCl4 (3ml/kg bw) in olive oil suspension (1:1 v/v) after 14 days pre-administration of the diet (4g/100g of sauce). They were grouped as follows I: control (basal diet), II: CCl4 + fresh sauce-supplemented diet, III: CCl4 + 1 month sauce-supplemented diet, IV: CCl4 + 2 months sauce supplemented diet, V: CCl4 + 3 months sauce-supplemented diet, VI: negative control (CCl4 + Basal diet). The effect on oxidative stress, inflammatory markers, and liver function parameters was observed. ROS, TNF-α, IL-6 and IL-1 were significantly p <0.05 reduced with a concomitant improvement in antioxidant status, and liver functions following pre-administration of the sauce-supplemented diet. This suggests the antioxidative potential of the sauce and that storage did not reduce the value of sauce produced from pepper and tomato fruits.

Keywords: Anti-inflammatory, Hepatotoxicity, Storage, oxidative stress

**1. Introduction**

*Capsicum* spp. (pepper) are popular vegetables belonging to the Solanaceae family. They are of about 90 genera and 2000 species of which five *C. chinense, C. annuum, C. frutescens C. pubescens, and C. baccatum* are well-cultivated (Liu et al., 2023). They are grown and consumed in tropical, subtropical, and temperate regions of Africa, Asia, America, and the Mediterranean basin (Sharifi-Rad et al., 2018). They are important food ingredients in cooking adding flavors to food and beneficial effects to its consumers (Jiang, 2019). Peppers are good sources of vitamins, provitamins, phenolics, carotenoids and can be consumed fresh or processed, as preserves, sauces, salad, soup or dehydrated powder. The presence of these compounds contributes to nutritional value, antioxidant activity and economic importance of the fruit. Similarly, *Lycopersicum esculentum* (tomato) forms an important component of food widely consumed and this is evident in the fact that many dishes local to different ethnicities around the world have it as an ingredient. They are adequately enriched with vitamins, pro-vitamins, minerals, secondary metabolites such as flavonoids, phytosterols, polyphenols, carotenoids, and other phenolic compounds such as quercetin, kaempferol, naringenin, lutein, as well as caffeic, ferulic and chlorogenic acids. This makes tomatoes valuable in health and disease management (Ali et al., 2020; Zhu et al., 2020).

Certain chemicals from the environment are toxic due to their metabolic activation into highly reactive substances. They can as such cause damage to different organs of our body. CCl4, a known toxicant to humans through inhalation, ingestion or dermal absorption causes cellular damage in multiple organs (Teschke, 2018). Metabolic activation through the cytochrome P450 pathways leads to toxicity in the kidney, testicle, brain, heart, lung and is particularly dangerous to the liver. They are as such used to induce hepatotoxicity in experimental animals. The inability of the body’s antioxidant defense to neutralize the toxic metabolites from CCl4 activation leads to oxidative stress (Afzal et al., 2023). The intake of exogenous antioxidants boosts the endogenous antioxidant defense and ameliorates the damage caused by oxidative stress through free radical scavenging, reductive reactions, and inhibiting the propagation of oxidative chain reactions amongst others (Zhou et al., 2016). Pepper and tomato fruits are functional foods due to the presence of health-promoting compounds and broad-spectrum pharmacological activities (Vignesh et al., 2024). They are incorporated into daily diets as spices, food supplements or additives by a quarter of the global population (Maji and Banerji, 2016).

A major limitation of pepper and tomato is their short shelf life because of their characteristic thin and dehydrated skin making them highly perishable. However, processing into easily stored products like sauce increases the shelf-life. Application of heat to food whether by cooking, boiling, oven drying and so on is a food processing method aimed at killing food pathogens for improved shelf life. Also, improved sensory qualities and compliance with regulatory standards are ensured. Processing technique may result in physicochemical changes that could result not only in sensory modifications but also in reducing the content or bioavailability of valuable antioxidants in the product. The goal of reducing the risk of microorganism contamination is crucial; however, it needs to be in balance with the retention of the quality attributes. The temperature and duration of heat treatment can influence the retention of phenolic compounds resulting in either loss or preservation of as many of the beneficial components as possible (Chaaban et al., 2017). Hence, optimization of the processing conditions is a key to balancing these aspects. This study evaluated the influence of processing and storage on the health benefits of sauce produced from two *Capsicum* spp (Pepper) and *Lycopersicum esculentum* (tomato) in experimental rats induced with hepatotoxicity by CCl4.

**2. Materials and methods**

*2.1. Sampling*

Fruits of *Capsicum chinense* (Habanero), *Capsicum annuum* (Cayenne) and tomato *Lycopersicum esculentum* were authenticated at the herbarium of the Ekiti State University, Ado Ekiti, Nigeria and given herbarium number UHAE 2023070, UHAE 2023071, UHAE 2023072 respectively. The pepper and tomato fruits were washed clean and blended into a sauce with the below composition.

**Table 1: Composition (g/100g) of pepper and tomato used for producing sauce**

|  |  |
| --- | --- |
| Fruits | g/100g |
| *Caspicum chinense*, Habanero | 25 |
| *Capsicum annuum*, Cayenne | 25 |
| *Lycopersicum esculentum* | 50 |
|  | 100g |

*2.2 Sample preparation*

*Heat application and storage*

The blended pepper and tomato sauce was boiled (93oC) for 10 mins and afterward transferred to a glass jar (450 ml). The glass jar was tightly covered with its lid and subsequently transferred to a boiling water bath for 30 minutes. The glass jars were stored for different periods on the shelf (1 month, 2 months, and 3 months).

*2.3 Animal study*

Wistar strain rats (Female; 150 - 200g) were procured from the College of Medicine, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. They were fed a standard rodent pellet (Pfizer) and water *ad libitum* for the period of acclimatization (7 days) under standard environmental conditions (12-h light and 12-h dark cycle). The criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the EU Directive for animal experiments (2010/63/EU) were followed for the handling of the animals. The experiment conducted according to the guidelines of the Declaration of Helsinki was carried out at the Biochemistry unit of the Department of Science Laboratory Technology, Ekiti State University, Ado Ekiti, Nigeria.

*2.4 Study Design*

2.4.1 Diet formulation and animal grouping

The sauce was supplemented in the diet to check the effect on rats induced with hepatotoxicity by CCl4. Skimmed milk, corn starch, vitamin premix, and vegetable oil were included in the sauce-supplemented diet as described in Adefegha and Oboh (2012)

**Table 2: Diet formulation**

Sample Basal diet (%) sauce supplemented diet

Skimmed milk 28 28

Corn starch 58 54

Vitamin premix 4 4

Vegetable oil 10 10

Sauce - 4

Experimental animals were administered CCl4 (3ml/kg body weight) in olive oil suspension (1:1 v/v) intraperitoneally according to Adeyanju et al. (2021). They were separated into six groups of five rats as presented below:

Group I: Normal control (basal diet + olive oil, vehicle)

Group II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

Group III: CCl4 (3ml/kg b.w) + 1 month sauce-supplemented diet

Group IV: CCl4 (3ml/kg b.w) + 2 months sauce-supplemented diet

Group V: CCl4 (3ml/kg b.w) + 3 months sauce-supplemented diet

Group VI: CCl4 (3ml/kg b.w) + basal diet

The sauce-supplemented diets were pre-administered for 14 days before the administration of CCl4. Clinical signs and changes in body weight were monitored during the study period.

*2.5 Biochemical Assays*

At the termination of the experiment after 24 hours post-CCl4 administration, blood samples were collected through cardiac puncture in an anti-coagulant (EDTA) bottle and centrifuged at 4000 rpm for 15 minutes for plasma retrieval. The plasma was evaluated for liver function and lipid profile parameters using standard protocols from Fortress Diagnostics Kit, U.K. Liver tissues were removed into 1.15% KCl, blotted with Whatman filter paper, weighed and subsequently homogenized in ice-cold buffer (0.1M, pH 7.4). The homogenization in a Teflon glass was done at approximately 1200 rpm with about ten up-and-down strokes. The homogenates were centrifuged for 10 min at 10,000 x g to yield discarded pellets and a post-mitochondrial fraction (supernatant) which was preserved at 4°C for analysis. Supernatants were assessed for inflammatory markers (TNF-α, IL-6, NF-kB) and reactive oxygen species (ROS). They were also evaluated for lipid peroxidation using a modified method reported by Tsikas (2017). They were also assessed for antioxidant markers (GSH, SOD, CAT, and GPx) using standard protocols.

2.5.1 Determination of Inflammatory Markers

TNF-α, IL1, and IL-6 were determined using Elabscience® ELISA Kit. They were measured and estimated according to the manufacturer’s instructions as stated in Adeleke et al., (2022).

2.5.2 Determination of ROS

According to the manufacturer's instructions, the ROS was determined using a Green Fluorescence ROS assay kit.

2.5.3 Estimation of Lipid Peroxidation.

The supernatant (300𝜇L) was pipetted into test tubes and the following reagents were added sequentially: 300𝜇L of Sodium dodecyl sulphate (8.1%), 500𝜇L of acetic acid (pH 3.4) and 500𝜇L of thiobarbituric acid (0.8% TBA). The mixture was incubated at 100oC for 1 hour, cooled and centrifuged at 3000 x g. The thiobarbituric acid reactive species (TBARS) produced was measured at 532 nm and expressed as µmol MDA/100g protein. Lipid peroxidation in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 105 M-1Cm-1

2.5.4 Determination of Antioxidants

SOD (Fridovich, 1989), Catalase (Claiborne, 1985), Glutathione peroxidase (Rotruck, 1973), and reduced glutathione (Beutler, 1963).

*2.6 Data analysis*

Replicate readings recorded during analysis were evaluated and expressed as mean ± standard deviation using graph pad prism 6.0. One-way ANOVA and Tukey’s test were statistically employed for multiple comparisons using 0.05 level of significance.

**3 Results and Discussion**

*3.1 Effect of pepper and tomato sauce-supplemented diet on liver function markers of rats induced with hepatotoxicity*

The present study revealed several beneficial properties of pepper and tomato sauce-supplemented diet. The effect on liver function of CCl4-induced hepatotoxic rats is presented in Table 3. CCl4 is known to induce liver damage in animal models and it significantly increased the activities of AST, ALT, ALP and LDH. In liver damage, these enzymes involved in amino acid metabolism are excreted outside the cell and their plasma level increases (Thabrew et al, 2017). These elevated levels are caused by damage to the cell membrane from oxidative stress. The effect of CCl4 is consistent with other studies demonstrating similar elevation following hepatic injury (Li et al., 2021; Algefare et al, 2024). Supplementation of the diet with the sauce reduced the activities of AST, ALT, ALP and LDH in the plasma which was initially altered by CCl4 administration. There was no significant (p > 0.05) difference across the treatment groups except that the group fed 3 months sauce-supplemented diet had elevated ALP and LDH activities. The protection of the membrane’s integrity can be linked to the antioxidative potential of the sauce produced from pepper and tomato which aligns with other studies showcasing the ability of pepper to significantly improve liver function in animal models (Koneru et al., 2018; Lee et al., 2022).

**Table 3: Effect of pepper and tomato sauce-supplemented diet on liver function markers of CCl4-induced hepatotoxic rats**

**Groups AST ALT ALP LDH**

I 49.6 ± 4.84a 51.7 ± 6.42a 53.4 ± 6.33a 74.6 ± 1.88a

II 52.7 ± 3.45a 52.0 ± 3.40a 52.4 ± 8.20a 72.0 ± 2.56a

III 57.4 ± 9.70b 53.8 ± 9.04a 59.7 ± 2.18b 72.7 ± 6.95a

IV 53.6 ± 4.87a 46.1 ± 3.69b 59.7 ± 0.67b 75.2 ± 7.34a

V 50.9 ± 7.02a 48.4 ± 5.98b 58.9 ± 3.85b 76.1 ± 1.95a

VI 60.2 ± 3.73c 58.4 ± 4.13c 61.1 ± 3.13b 80.1 ± 5.53b

Values represent mean ± standard deviation (n = 5)

Values with the same letter down columns are not significantly different (p > 0.05)

I: Normal control (basal diet)

II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

III - V: CCl4 (3ml/kg b.w) + 1 month, 2 months, 3 months sauce-supplemented diet respectively

VI: CCl4 (3ml/kg b.w) + basal diet

*3.2 Effect of pepper and tomato sauce-supplemented diet on lipid profile of rats induced with hepatotoxicity*

Furthermore, the results obtained in this study clearly show that the sauce-supplemented diet has a lowering effect on plasma total cholesterol, low density lipoprotein (LDL-C), and triglycerides with a concomitant increase in the plasma level of high density lipoprotein (HDL-C) as shown in Table 4. Lipoproteins are important body chemicals as their ratio is a determinant of cardiovascular function. There is substantial evidence that lowering the total cholesterol, particularly LDL-C and improving the HDL-C level will lead to a reduction in the incidence of coronary heart disease (Adeyemi et al., 2008). The lipid profile of rats fed the sauce-supplemented diet was greatly improved with the 2-month sauce showing better lipid-lowering properties than the other groups. Experimental rats fed the 2 months sauce-supplemented diet had 74.3 mg/dl HDL and 54.4 LDL as compared to the normal control (70.0 mg/dl, 55.3 mg/dl) and CCl4 control (54.8 mg/dl, 56.6 mg/dl). The HDL and LDL levels following administration of the sauce-supplemented diet suggest the improvement of lipid transport from the blood to the liver for metabolism. The implication of this is the prevention of lipid deposits in the arterial wall and the improvement of cardiovascular health. The improvement of cardiovascular functions as a result of the lipid-lowering effect of pepper and tomato might be accounted for by their richness in carotenoids, vitamins, and flavonoids (Helen and Vijayammal, 2016).

**Table 4: Effect of pepper and tomato sauce-supplemented diet on lipid profile of CCl4-induced hepatotoxic rats (mg/dl)**

**Groups HDL TRIG TC LDL**

I 70.0 ± 11.3a 50.9 ± 6.50a 52.8 ± 4.03a 55.3 ± 3.46a

II 70.5 ± 7.94a 52.5 ± 6.20a 51.6 ± 2.38a 53.5 ± 3.77a

III 62.1 ± 3.76b 58.6 ± 2.80b 55.6 ± 5.19a 54.2 ± 0.79a

IV 74.3 ± 11.8a 52.1 ± 4.60a 53.2 ± 2.76a 54.4 ± 1.98a

V 57.1 ± 4.81b 49.4 ± 3.35a 57.2 ± 2.70b 55.9 ± 1.32a

VI 54.8 ± 6.06c 59.5 ± 2.76b 58.4 ±2.83b 56.6 ± 2.97a

Values represent mean ± standard deviation (n = 5)

Values with the same letter down columns are not significantly different (p > 0.05)

I: Normal control (basal diet)

II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

III - V: CCl4 (3ml/kg b.w) + 1 month, 2 months, 3 months sauce-supplemented diet respectively

VI: CCl4 (3ml/kg b.w) + basal diet

*3.3 Effect of pepper and tomato sauce-supplemented diet on inflammatory markers of rats induced with hepatotoxicity*

CCl4 administration induced hepatotoxicity in rats with a significant increase in TNF-α, IL-1 and IL-6. The pretreatment of rats with the sauce-supplemented diet significantly reduced (p < 0.05) the values to comparable levels with the normal control and way better than the CCl4 control (Figure 1). The rats fed 1-month sauce-supplemented diet had the lowest TNF-α (13.66 pg/ml) and IL-6 (141.1 pg/ml) levels while the 2-month sauce-supplemented diet had the lowest IL-1 level (25.87 pg/ml) as compared to the normal control (13.97, 26.88, 149.8 pg/ml) and CCl4 control (16.96, 31.19, 158.0 pg/ml) for TNF-α, IL-1, and IL-6 respectively. CCl4 can activate TNF-α, IL-1, and IL-6 which are inflammatory mediators contributing to liver injury like fibrosis, necrosis, or apoptosis (Hur et al., 2012; Prasad et al., 2014).



**Figure 1: Effect of pepper and tomato sauce-supplemented diet on inflammatory markers of CCl4-induced hepatotoxic rats**

Bars represent mean ± standard deviation (n = 5). \*Values significantly different from normal control p < 0.05. **#**Values significantly different from CCl4 control p < 0.05

I: Normal control (basal diet)

II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

III - V: CCl4 (3ml/kg b.w) + 1 month, 2 months, 3 months sauce-supplemented diet respectively

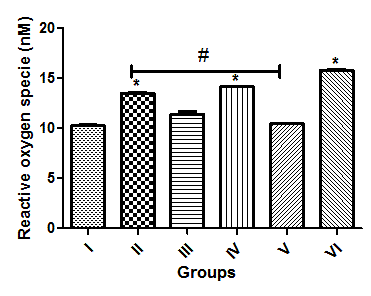
VI: CCl4 (3ml/kg b.w) + basal diet

Importantly, it is well-documented that phytochemicals or dietary bioactive compounds from functional foods have beneficial outcomes against inflammation and are considered potential candidates against pathological processes mediated by inflammation (Prasad et al., 2014). Data from this study suggest that *Capsicum* spp and *Lycopersicum esculentum* may contain major bioactive compounds involved in their anti-inflammatory activity. There is evidence of significant concentrations of total phenols, capsaicinoids, ascorbic acid and other phenolic compounds in pepper and tomato fruit. These compounds in pepper and tomato have anti-inflammatory properties making the sauce produced from them active as an antiinflammatory agent against CCl4-induced toxicity (Antonious et al., 2006; Zheng et al., 2017).

*3.4 Effect of pepper and tomato sauce-supplemented diet on oxidative stress markers*

*of rats induced with hepatotoxicity*

There was an elevation of reactive oxygen species (Figure 2) and lipid peroxidation (Figure 3) in the liver tissues of CCl4-induced hepatotoxic rats. This demonstrated the toxic effect of CCl4 and the consequent tissue damage arising from oxidative stress. The formation of CCl3˙ from CCl4 by the action of CYP enzymes results in the reaction of the previous with oxygen to give a highly reactive radical, CCl3 OO˙ (Brattin et al., 2015). Supplementation of the diet with pepper and tomato sauce reduced the ROS level (13.47, 11.38, 14.18, 10.48 nM) for fresh and stored sauce when compared to the normal control (10.25 nM) and CCl4 control (15.85 nM).

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**Figure 2: Effect of pepper and tomato sauce-supplemented diet on reactive oxygen species of CCl4-induced hepatotoxic rats**

Bars represent mean ± standard deviation (n = 5). \*Values significantly different from normal control p < 0.05. **#**Values significantly different from CCl4 control p < 0.05

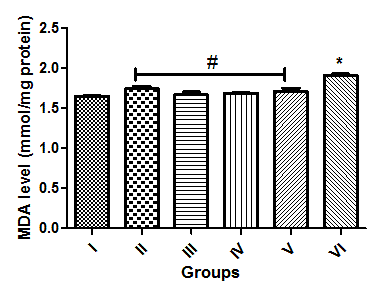
I: Normal control (basal diet)

II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

III - V: CCl4 (3ml/kg b.w) + 1 month, 2 months, 3 months sauce-supplemented diet respectively

VI: CCl4 (3ml/kg b.w) + basal diet

Furthermore, CCl4 administration increased the MDA levels compared to the control group but decreased significantly in groups fed the sauce-supplemented diet before CCl4 administration compared to the CCl4 control group. Lipid peroxidation is a hallmark of oxidative damage and a convenient method to monitor oxidative stress (Viani et al., 2021). Pepper and tomato sauce-supplemented diet was able to prevent oxidative stress which is evident in a significant reduction of ROS and lipid peroxidation compared to the control and CCl4 control group.

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**Figure 3: Effect of pepper and tomato sauce-supplemented diet on lipid peroxidation of CCl4-induced hepatotoxic rats**

Bars represent mean ± standard deviation (n = 5). \*Values significantly different from normal control p < 0.05. **#**Values significantly different from CCl4 control p < 0.05

I: Normal control (basal diet)

II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

III - V: CCl4 (3ml/kg b.w) + 1 month, 2 months, 3 months sauce-supplemented diet respectively

VI: CCl4 (3ml/kg b.w) + basal diet

*3.5 Effect of pepper and tomato sauce-supplemented diet on the antioxidant status of rats induced with hepatotoxicity*

The administration of CCl4 resulted in the depletion of the antioxidant system (SOD, CAT, GPx, and GSH (Table 5). A similar decrease in the activity of hepatic antioxidant enzymes by CCl4 has been previously reported, linking the hepatocellular toxicity of CCl4 to oxidative stress damage (Li et al., 2021)*.* The CCl4 administration resulted in a significant decrease in SOD activity (51.78) compared to all other groups. The highest activity was recorded for the 3 months sauce-supplemented diet group (58.93) showing that storage for 3 months did not alter the potency of the sauce in terms of its superoxide dismutase activity. On the other hand, GSH decreased significantly in the CCl4 group (47.77) compared to the control group (56.80); co-administration of the sauce-supplemented diet with CCl4 caused the GSH to improve greatly with the group fed 2 months sauce-supplemented diet having the highest GSH level (59.52). Similar trends were observed for GPx and catalase activity. The group fed a fresh sauce-supplemented diet had the highest GPx activity (40.25) and the group fed a 3 months sauce-supplemented diet with the highest catalase activity (59.67). Bioaccumulation of CCl4 in organs such as the liver has been shown to result in depletion of their enzymatic and non-enzymatic antioxidant system leading to redox imbalance (Arisawa et al., 2007). The depletion in the antioxidant enzymes could be attributed to the exhaustive mobilization of these enzymes to scavenge the reactive CCl3., CCl3O and other reactive species associated with CCl4 hepatocellular toxicity (Gul et al., 2015). This decrease could result in the deficiency of necessary antioxidant enzymes to prevent the cell from being assaulted by reactive oxygen species and free radicals subsequently resulting in hepatocellular damage.

**T****able 5: Effect of pepper and tomato sauce on antioxidant status of CCl4-induced hepatotoxic rats**

**Groups SOD GSH GPx CAT**

I 55.04 ± 3.08b 56.80 ± 3.29b 39.35 ± 3.02a 60.60 ± 6.58a

II 55.55 ± 5.02b 56.46 ± 5.74b 40.25 ± 3.80a 49.88 ± 1.38b

III 49.23 ± 2.20c 56.50 ± 5.92b 41.37 ± 8.12a 58.42 ± 4.08a

IV 57.35 ± 9.90a 59.52 ± 2.79a 40.10 ± 3.52a 49.15 ± 4.62b

V 58.93 ± 3.97a 56.47 ± 3.84b 37.94 ± 5.16b 59.67 ± 4.24a

VI 51.78 ± 2.54c 47.77 ± 7.45c 38.44 ±4.60b 47.19 ± 3.65c

Values represent mean ± standard deviation (n = 5)

Values with the same letter down columns are not significantly different (p > 0.05)

I: Normal control (basal diet)

II: CCl4 (3ml/kg b.w) + fresh sauce-supplemented diet

III - V: CCl4 (3ml/kg b.w) + 1 month, 2 months, 3 months sauce-supplemented diet respectively

VI: CCl4 (3ml/kg b.w) + basal diet

The ultimate benefit of antioxidant markers is to defend the body against oxidants converting them into non-toxic stable compounds. SOD dismutase superoxide anion (O2-) into hydrogen peroxide (H2O2) and molecular oxygen; catalase and glutathione peroxidase destroy hydrogen peroxide to produce water and oxygen; glutathione is a chief antioxidant molecule modulating physiological levels of ROS. The activities of SOD, catalase, GPx and level of GSH were maintained at normal levels when compared with the control implying that the sauce significantly enhanced the antioxidant capacity. The preservation of the antioxidant enzyme system also provides more supporting evidence for the in-vivo antioxidant potential of *Capsicum spp* and tomato (*Lycopersicum esculentum*).

**Conclusion**

Processing improved the shelf life of the pepper and tomato sauce up to three months evaluated in this study. The sauce prevented oxidative stress through its antioxidant and anti-inflammatory pathways to protect the liver against damage brought on by CCl4. Although some high benefits were observed in the 3 months sauce-supplemented diet, there were also some declines. This suggests a possible loss of antioxidants and anti-inflammatory potential for a longer storage period.

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