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# Effectiveness of organic tomato (*Solanum lycopersicum L.*) extracts added to yeast Cell Culture against $H_2O_2$ Toxicity

## Eficácia de extratos de tomate orgânicos adicionados à cultivos de células de levadura contra la toxicidad por $H_2O_2$

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## ABSTRACT

Growing tomatoes in season and organically are an important factor in terms of carrying higher levels of antioxidant molecules. This study aimed to demonstrate the protective effect of extracts obtained from naturally grown tomatoes, which are widely used as food, against oxidative damage caused by hydrogen peroxide on yeast cells (*Saccharomyces cerevisiae* L.). Lipophilic vitamins, phytosterol content, gluathione and oxidised gluathione content were analyzed by Shimadzu brand high performance liquid chromatography, while fatty acid analysis was performed by Shimadzu GC 2010 Plus Gas Chromatography. Protein content was analyzed spectrophotometrically. The study groups were designed as control, hydrogen peroxide, tomato and tomato + hydrogen peroxide. In all groups added with hydrogen peroxide and tomato extract compared to the hydrogen peroxide group; it was observed that vitamin D2, α-Tocopherol, β-Sitosterol and Stigmasterol contents increased significantly. Again, it was observed that ergosterol level increased significantly in the D + hydrogen peroxide group compared to the hydrogen peroxide group. When the fatty acid analysis results were examined, it was determined that in all groups added to the cultured yeast with hydrogen peroxide and tomato extracts (except the B + hydrogen peroxide group), there were increases in 16:0, 16:1, n-7, 17:0, 17:1 and 18:0 fatty acid levels compared to the hydrogen peroxide group. On the other hand, when its effect on protein and gluathione levels was examined, more significant increases were determined in all groups with tomato extract addition compared to the hydrogen peroxide group. At the end of the study, it was observed that tomato extracts had positive effects against hydrogen peroxide toxicity in yeast cells, although at different levels in terms of fatty acid, vitamin and phytosterol contents.

**Key words:** Fatty acids; vitamin; phytosterol.

## RESUMEN

El cultivo de tomates en temporada y orgánicamente es un factor importante para obtener frutos con mayor propiedades antioxidantes. Este estudio tuvo como objetivo demostrar el efecto protector de los extractos obtenidos de tomates de cultivo natural, ampliamente utilizados como alimento, contra el daños oxidativo causado por el peróxido de hidrógeno en células de levadura (*Saccharomyces cerevisiae* L.). El contenido de vitaminas lipofílicas, fitosterol, glutatión y disulfuro de glutatión se analizaron mediante la cromatografía líquida de alta resolución Shimadzu, mientras que el análisis de ácidos grasos se realizó mediante cromatografía de gases Shimadzu GC 2010 Plus. El contenido de proteínas se analizó espectrofotométricamente. Los grupos de estudio se diseñaron como control, peróxido de hidrógeno, tomate y tomate + peróxido de hidrógeno.En todos los grupos adicionados con peróxido de hidrógeno y extracto de tomate, en comparación con el grupo peróxido de hidrógeno, se observó un aumento significativo en los niveles de vitamina $D_2$, α-tocoferol, β-sitosterol y estigmasterol. De nuevo, se observó un aumento significativo en el nivel de ergosterol en el grupo D + peróxido de hidrógeno en comparación con el grupo peróxido de hidrógeno. Al examinar los resultados del análisis de ácidos grasos, se determinó que en todos los grupos que añadieron extractos de tomate y peróxido de hidrógeno a la levadura cultivada (excepto el grupo B + peróxido de hidrógeno), se observaron aumentos en los niveles de ácidos grasos 16:0, 16:1, n-7, 17:0, 17:1 y 18:0 en comparación con el grupo peróxido de hidrógeno. Por otro lado, al examinar su efecto sobre los niveles de proteína y glutatión, se observaron aumentos más significativos en todos los grupos con adición de extracto de tomate en comparación con el grupo peróxido de hidrógeno. Al final del estudio, se observó que los extractos de tomate tuvieron efectos positivos contra la toxicidad del peróxido de hidrógeno en las células de levadura, aunque con diferentes niveles en cuanto al contenido de ácidos grasos, vitaminas y fitosterol.

**Palabras clave:** Acidos grasos; vitamina; fitoesterol.

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## INTRODUCTION

The oxygen molecule, which is quite reactive, can be chemically reduced to reactive oxygen types (ROS), which are other smaller materials that have high reactivity [1]. These are radical types such as superoxide anion ($O_2$) and hydroxyl radicals (OH) and moreover non-radical oxygen types such as hydrogen peroxide ($H_2O_2$) and singlet oxygen ($O_2$) [2]. Additionally, ROS products, which may be caused by environmental factors (such as UV, ionizing radiation, pollutants, and heavy metals, cigarette), form a phenomenon with them being prooxidants and their ability to neutralize ROS products known as imbalance [2 , 3].

On the other hand, at the conclusion of this, injuries occur to cellular components such as membranes, proteins, lipids, nucleic acids [4]. Antioxidants, on the other hand, can turn ROS types that damage cells into products with little to no toxicity by reducing them. Antioxidant mechanisms, similarly, to how they are created by body cells, can also be taken from the outside through nutrition. Antioxidants, natural antioxidants foremost, that are taken from nutrition (fruits and vegetables) and have protective properties against free radicals are vitamins (A, C, E), flavonoids, carotenoids, and polyphenols. Fruit antioxidants are known to protect tissues against stress and illnesses [5].

Yeast cells, being the foremost producers used worldwide in the biotechnology branch, are used more relatively to other industrial microorganisms. Especially in terms of the fact that the *Saccharomyces cerevisiae* L. yeast cell demonstrates macromolecular similarities to mammal cells, that its usage in nutritional goods is reasonable, that its price is appropriate, and that it is an easy microorganism to acquire, it has become a microorganism used more often in studies [6]. In addition, the *Saccharomyces cerevisiae* genome contains 16 chromosomes, and the fact that its entire genome has been sequenced provides great opportunities for studying various cellular processes [7]. Other reasons for choosing *S. cerevisiae* as a model organism are easy modification of its genetic structure, easy isolation of mutants, and the presence of haploid and diploid cell types [8]. Thanks to these features, *S. cerevisiae* yeast is widely used in the elucidation of many unknowns such as cell cycle, protein secretion, membrane formation, cytoskeleton function, cell differentiation, aging, gene function and chromosome structure [9]. Due to all these characteristics, *Saccharomyces cerevisiae* was preferred in this study [10].

*Saccharomyces cerevisiae* L. yeast cells are known as the most widely accepted eukaryotic cell models for studying xenobiotic and other toxic substance effects [11] because they exhibit macromolecular similarities to mammalian cells [6] and resistance to toxic substances. Due to these similarities with animal cells, they were chosen in this study to serve as a precursor for animal model studies.

Tomatoes (*Solanum lycopersicum* L.) are plants that have over 3,000 types, belong to the Solanaceae family, can be seen yearly or perennially in nearly every location of the world, have closed seeds and are dicotyledonous, and are believed to have originated from South America [12]. Alongside the fact that it was first farmed in Europe, countries such as Türkiye, China, India, the USA, and Egypt have left Europe behind in terms of agriculture in modern days and are currently ranked in first place for their product amounts [7].

This nutrition source that has a very important place in human nutrition, has rich natural antioxidants and micronutrient content including carotenoids (lycopene), ascorbic acid, potassium, folate, and phenolic compounds. Moreover, the chemical makeup of this fruit can vary depending on growth techniques, as well as cultivation and storing conditions [8]. On the other hand, the phenolic compounds in tomatoes have been comprehensively characterized by various tomato genotypes. Alongside flavonoids such as rutina, quercetin, naringenin, calconaringenin, and kaempferol derivatives, they have been reported as the main phenolic compounds in chlorogenic acids and related components (hydroxycinnamates) [13]. It has been reported that tomatoes are rich in many nutrients that are very important for human health, including vitamins (C, E, A, B1, B2 and folic acid), minerals (potassium, phosphorus, calcium, iron and magnesium), phenolic antioxidants, sugars, amino acids, lycopene, carotenoids, phenolic and dietary fibers [14].

The nutritional benefits of tomatoes are largely attributed to their phytochemical composition, and they have been reported as a functional food that protects people against chronic degenerative diseases such as diabetes, microvascular complications, viral diseases, and cancers [15]. The most studied carotenoid in tomatoes [16] is lycopene, which has been reported as a powerful free radical scavenger that protects humans against cellular oxidative damage [17]. And previous studies have reported that whole tomatoes offer a superior protective effect, especially compared to lycopene supplementation alone [18]. Song et al. reviewed 14 relevant studies and found a significant inverse association between lycopene intake and coronary heart disease [19], while another meta-analysis reviewed 25 studies and reported that high lycopene consumption and serum lycopene concentrations reduced overall mortality by 37 %, cardiovascular disease by 14 %, and stroke risk by 23 % [20]. Furthermore, pathological impairments due to Alzheimer's disease, Parkinson's disease, and cerebral ischemia have been shown to improve with lycopene and tomato extract in both in vitro and animal studies [21].

Studies conducted on tomatoes until now have been about determining the antioxidant content of tomatoes or whether beneficial microorganisms have properties that benefit the encouragement of this plant's growth [22]. However, there are few studies examining the effects of tomato components on yeast cell growth [23 , 24]. With this study, by conducting the analyses of the biochemical effects of tomato extracts that were added to the culture of *Saccharomyces cerevisae* L. with oxidative damage formed by $H_2O_2$ on some molecules (Protein, gluathione (GSH), oxidised gluathione (GSSG), fatty acids, and A, D, E, K vitamins), studying whether or not the phytochemicals in tomatoes are protective against the degenerative effect caused by hydrogen peroxide ($H_2O_2$) was aimed.

## MATERIALS AND METHODS

### Preparation of the *Saccharomyces cerevisiae* cultivation environment

Disolve 20 g peptone water, 20 g yeast extract and 40 g D-Glucose in 1 L of distilled water, within an ultrasonic water bath for 20 minutes (min), labelling and equal distribution to 250 mL bottles was conducted. 200 mL of growth medium was added to each bottle, after which the autoclave (150L Vertical Autoclave – SUS304, 134 °C, 32 PSI, USA) was programmed to 121 °C at 15 atm pressure and it was sterilized for 15 min. The incubator (MEMMERT IN110, USA) was set to 30 °C and the mixture was left for incubation for 72 hours (h) [25].

### Gathering of herbal materials and preparation of plant extracts

In this study, tomato samples grown organically in the summer season were collected from 4 different regions of Elazığ (Türkiye). These regions were determined to be Baskil (B), Cip (C), Doğukent (D), and Sivrice (S). And sample materials were separately gathered while fresh and brought to the labs in which the experiments are conducted. 100 g of each tomato sample was weighed (Tree HRB-S 313 Stainless Steel Precision Balance, USA), homogenized (IKA T 25 Digital ULTRA-TURRAX, Germany) with the help of 250 mL of 85 % methanol in a blender and later filtered with the use of filtering paper (Merck, 0.2 µm). Disolved materials were evaporated and separated using a rotary evaporator (Merck, Heidolph Rotary Evaporator, Germany) at 50 °C. The resulting portion was dissolved in sulfoxide (DMSO) and completed to 50 mL of volume. 1 mL was taken from this mixture, added to *S. cerevisiae* cultivations (200 mL), and left at 30 °C for incubation [26].

The groups were formed as follows after the preparation of the growth medium environment:

1- Control Group (C): This group, the medium environment, was prepared to be 200 mL of distilled water, 2 g of yeast extract (1 %, w/v) (Biolife, Italy), 4 g bacto peptone (2 %, w/v) (Biolife, Italy) and 4 g glucose (2 %, w/v) (Merck, Germany).

2-Hydrogen Peroxide Group ($H_2O_2$): This group containing 200 mL of distilled water, 2 g of yeast extract, 4 g bacto peptone and 4g of glucose was prepared. Moreover, 100 µL of $H_2O_2$ solution (35 %, Sigma Aldrich, USA) was added at the start of the incubation process.

3-Groups with Added Tomato Extracts: This group containing 200 mL of distilled water, 2 g of yeast extract, 4 g bacto peptone and 4 g of glucose and 1 mL of tomato extract was added to the medium environment (B, C, D, S groups).

4-Groups with Added Tomato Extract + $H_2O_2$: This group containing 200 mL of distilled water 2 g of yeast extract, 4 g bacto peptone and 4 g of glucose was used and 1mL of tomato extract alongside 100 of µL $H_2O_2$ solution were added at the start of the incubation process (B + $H_2O_2$, C + $H_2O_2$, D + $H_2O_2$, S + $H_2O_2$ groups).

After the inoculate of yeast cells under sterile conditions into the cultivation environment, the cultures were left at 30 °C for 72 h to incubate. At the end of this process, after the measurement of cell densities (METTLER TOLEDO Densito 30 PX, Germany) of the cultures under laboratory conditions at 600nm, centrifuge (Hettich Zentrifugen D-78532 Tuttlingen, Germany) at 100 cps for 5 s at + 4 °C was conducted, and the cells were collected.

After the collection of cell pellets, their wet weights were determined. After the cell pellets were homogenized (IKA T 25 Digital ULTRA-TURRAX, Germany) with 20 mM Tris HCl-base (pH = 7.4) and 20 mM (10 mL) ethylenediaminetetraacetic acid (EDTA) mixture and centrifuged, the measurements of the supernatant portion alongside the GSH and protein were conducted, while the remaining pellet portion was homogenized with 10 mL of the n-hexane/isopropanol mixture at a ratio of 3/2 (v/v) and used for the analyses of vitamins (A, D, E, K) and sterols (ergosterol) [22].

### The determination of total protein

Intracellular total protein measurements of samples obtained from cell pellets were conducted spectrophotometrically (Shimadzu UV mini 1240 brand, USA) according to the method defined by Lowry *et al.* [27]. After adding Lowry solvent, the samples were allowed to stand for 10 min. Subsequently, the Folin reactive distilled with water at a ratio of 1/1 (v/v) was added, and the samples was left to rest for 30 min under a dark environment. At the conclusion of the process, total protein calculation was made according to the calibration curve created from pure protein (albumin) and verified at 750 nm. And the calculation was made by performing 5 trials from each group. The resulting conclusions were expressed as mg/g.

### The determination of glutathione and glutathione disulfide values with the High performance liquid chromatography device

One mL of the remaining sample from the supernatant was taken and 10 % Perchloric Acid was added (to dissolve the proteins), followed up by the centrifuge process at 100 cps for 5 s, newly formed supernatant was transferred to the autosampler vials. The analyses were conducted in the HPLC (High performance liquid chromatography) device. To this end, 50 mmol of the $NaClO_4$ (0,1 % $H_3PO_4$) tampon was used as the mobile phase while the ODS 3 HPLC column (150 mm x 4.6, 5 µm) was used as the column. The mobile phase flow rate was set to 1,0 mL/min, while the DAD detector's wavelength was set to 215 nm. The resulting values were calculated using the LabSolutions 5.67 (Kyoto Japan) program [28].

### Extraction of lipids and vitamins

The extraction process of the fatty acids, A, D, E, and K vitamins, ergosterol, and other sterols belonging to the obtained sample mixtures was conducted according to the method defined by Hara and Radin [29]. To this end, samples were homogenized (IKA T 25 Digital ULTRA-TURRAX, Germany) for 30 sec. with the hexane-isopropanol mixture at a ratio of 3:2 (v/v), and the obtained homogenate was centrifuged at 83,33 cps for 10 s. Finally, the analyses of the fatty acids, A, D, E, and K vitamins, ergosterol and other sterols of this obtained supernatant portion was conducted.

### Determination of A, D, E, and K vitamins alongside Ergosterol content with the High performance liquid chromatography device

After the addition of 10 % methanolic potassium hydroxide (KOH) solution to the samples reserved for this determination, they were vortexed (DLAB MX-S, China). Afterwards, this mixture was left to rest at 85 °C for 15 min. After this time, the tubes were left to cool at room temperature and then mixed with water.

For the non-saponified lipophilic molecules, on the other hand, 5 mL of hexane was used alongside the extract. The hexane phase utilized for this, nitrogen gas was utilized, and it was evaporated. After the remaining residue was dissolved with 1,0 mL (% 60 + % 40 v/v) of acetonitrile/methanol mixture, it was transferred to autosampler vials. Analyses were conducted using the Shimadzu brand HPLC device. The acetonitrile/methanol (% 60+% 40, v/v) mixture was used as the mobile phase for this measurement [30].

### Preparation of fatty acid methyl esters and analysis with Gas Chromatography

To determine the fatty acid composition, samples were shaken in a vortex device (DLAB MX-S, China) after adding 2 % methanolic sulfuric acid. Afterwards, this obtained mixture was left to be methylated at 55 ºC for 15 h. To protect the lipid fraction against oxidation; BHT (Butylated hydroxytoluene) (0.001mL) was used in lipid isolation. And after the 15-h duration, the tubes were cooled at room temperature and properly mixed with the addition of 5 % sodium chloride (NaCl). And this way, the formed fatty acid methyl esters were extracted with hexane. The hexane phase formed at the end of this process, on the other hand, was taken with a pipette, treated with 2 % potassium bicarbonate ($KHCO_3$), and left for phase separation for 4 hours [31]. Methylation esters were dissolved in 1 mL of n-hexane and transferred to 2 mL capped autosampler vials and analyzed using Shimadzu GC-10 Gas Chromatography. A SP-2380 capillary GC column (L × ID: 30m × 0.25mm, df: 0.20 µm) was used for analysis (90 % biscyanopropyl/10 % cyanopropylphenyl siloxane).

### Statistical analysis

Statistical Analyses were conducted with the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) package program. The comparisons between the control and experimental groups were conducted with the ANOVA (Analysis of Variance; one-way ANOVA) test, while inter-group comparisons were conducted with the LSD test.

## RESULTS AND DISCUSSION

### Protein, GSH and GSSG levels

The changes in protein, GSH, and GSSG values of *S. cerevisiae* pellets with added tomato extracts relative to the control and $H_2O_2$ groups have been demonstrated in TABLE I. According to our findings, the total protein amount, relative to the control group, demonstrated decreases in the $H_2O_2$ group ($P < 0.01$) while the S + $H_2O_2$ group demonstrated a mild increase ($P < 0.05$). On the other hand, no differences in the other groups relative to the control group were found ($P > 0.05$). Groups which were given tomato extracts, when compared to the $H_2O_2$ group, demonstrated higher protein levels than the $H_2O_2$ group ($P < 0.05$, $P < 0.01$). When the GSH level is compared to the control group, it was demonstrated that although it decreased in code B and C groups with added extract ($P < 0.05$), the D and S groups demonstrated significant increases ($P < 0.01$). Alongside this finding, when the groups with added extract are compared to the $H_2O_2$ group, all groups with added extract demonstrated increases of varying levels in GSH levels compared to the $H_2O_2$ group ($P < 0.05$, $P < 0.01$, $P < 0.001$). When the GSSG group is compared to the control group, the B group demonstrated distinct ($P < 0.001$), the C group demonstrated no statistical differences, while other groups demonstrated partial decreases ($P < 0.05$). When the groups with added extract are compared to the $H_2O_2$ group, it was determined that the $H_2O_2$ group demonstrated distinct decreases ($P < 0.001$) (TABLE I).

**TABLE I**
**The variation of intracellular total protein and GSH levels in *S. cerevisiae* pellets supplemented with tomato (*Solanum lycopersicum L.*) extracts compared to the control and $H_2O_2$ group**

| Groups | Total protein (mg/g) | GSH (µg/g) | GSSG (µg/g) |
|---|---|---|---|
| Control | 1.41 ± 0.04 | 135.34 ± 0.98 | 29.03 ± 1.36 |
| B | 1.47 ± 0.12 $^{c}$ | 101.52 ± 2.07 $^{b}$ | 55.41 ± 1.11 $^{d}$ |
| C | 1.52 ± 0.02 $^{a}$ | 111.41 ± 1.97 $^{b}$ | 31.59 ± 1.33 $^{a}$ |
| D | 1.41 ± 0.07 $^{a}$ | 164.78 ± 2.64 $^{c}$ | 21.21 ± 1.21 $^{b}$ |
| S | 1.55 ± 0.07 $^{a}$ | 171.31 ± 3.95 $^{c}$ | 23.25 ± 1.08 $^{b}$ |
| $H_2O_2$ | 1.18 ± 0.03 $^{b}$ | 101.52 ± 3.71$^{b}$ | 48.18 ± 0.85$^{c}$ |
| B+ $H_2O_2$ | 1.32 ± 0.04 $^{a, b}$ | 124.89 ± 1.11 $^{a, b}$ | 24.32 ± 0.49 $^{a, d}$ |
| C+ $H_2O_2$ | 1.34 ± 0.03 $^{a, b}$ | 149.21 ± 3.99 $^{a, c}$ | 30.51 ± 0.74 $^{a, d}$ |
| D+ $H_2O_2$ | 1.32 ± 0.05 $^{a, b}$ | 139.18 ± 3.68 $^{a, b}$ | 19.32 ± 0.32 $^{b, d}$ |
| S+ $H_2O_2$ | 1.59 ± 0.07 $^{b, c}$ | 167.49 ± 5.61 $^{c, d}$ | 27.96 ± 0.88 $^{a, d}$ |

*2nd letterings express the comparison of added tomato extracts with the $H_2O_2$ group.

All of the specified protein contents of tomatoes have also always been a topic of interest in medical terms [32]. In the groups we formed to observe the impact of tomato extracts on the protein content of yeast cells; determined that compared to both the control group and the $H_2O_2$ group, all groups demonstrated increases in total protein content. When studies in literature are inspected, it was reported that all studied extracts' antioxidant activities (such as glutathione, SOD, peroxidase, catalase, ABTS, DPSS, and NO and DPHH free radical cleaning activities) are positively correlated with flavonoid compounds' amounts [33]. Similarly to these studies, especially in tomato extracts compared to the $H_2O_2$ group in our study; it was found that GSH, which is an indicator of antioxidant, increased, alongside which GSSG values, a decrease in which is considered to be positive, demonstrated decreases compared to the $H_2O_2$ group.

### Fatty acid content

The amounts of fatty acids obtained by gas chromatography are shown in TABLE II.

In the *S. cerevisiae* pellets of groups formed only with tomato extract; It determined that palmitic acid (C16:0), palmitoleic acid (C16:1, n-7), stearic acid (C18:0), oleic acid (C18:1 n-9), and linoleic acid (18:2 n-6) were observed in high amounts, while the levels of fatty acids such as lauric acid (C12:0), myristic acid (C14:0), heptadecanoic acid (17:0), heptadecenoic acid (C17:1), and linolenic acid (C18:3 n-3) were determined to have low amounts. When this difference in fatty acid levels among the groups is inspected, it was determined that while distinct decreases in the palmitic acid levels were observed in the B group ($P < 0.001$), it was determined that the decreases observed in groups C, D, and S were not statistically significant (TABLE II).

**TABLE II**
**The variation of fatty acid percentage in *S. cerevisiae* pellets supplemented with tomato (*Solanum lycopersicum L.*) extracts compared to the control group**

| Fatty acids | Experimentation Groups | | | | | |
|---|---|---|---|---|---|---|
| | Control | $H_2O_2$ | B | C | D | S |
| **C14:0** | 1.93 ± 0.13 | 1.11 ± 0.11 | 0.41 ± 0.03 $^{d}$ | 0.88 ± 0.02 $^{d}$ | 0.74 ± 0.04 $^{d}$ | 0.67 ± 0.05 $^{d}$ |
| **C16:0** | 28.74 ± 0.36 | 25.77 ± 0.76 $^{b}$ | 23.68 ± 0.33 $^{d}$ | 27.96 ± 0.99 $^{a}$ | 27.77 ± 0.45 $^{a}$ | 26.56 ± 0.91 $^{a}$ |
| **C16:1 n-7** | 18.66 ± 0.34 | 15.13 ± 0.24 $^{c}$ | 10.07 ± 0.27 $^{d}$ | 19.19 ± 0.55 $^{a}$ | 17.67 ± 0.82 $^{a}$ | 14.14 ± 0.34 $^{c}$ |
| **C17:0** | 0.62 ± 0.03 | 0.55 ± 0.03 | 0.44 ± 0.02 $^{b}$ | 0.82 ± 0.07 $^{b}$ | 0.76 ± 0.04 $^{b}$ | 0.69 ± 0.08 $^{a}$ |
| **C17:1** | 0.47 ± 0.03 | 0.44 ± 0.04 | 0.26 ± 0.01 $^{c}$ | 0.59 ± 0.06 $^{a}$ | 0.53 ± 0.06 $^{a}$ | 0.43 ± 0.02 |
| **C18:0** | 15.11 ± 0.51 | 21.30 ± 0.51$^{d}$ | 17.04 ± 0.47 $^{b}$ | 18.41 ± 0.49 $^{c}$ | 22.83 ± 0.74 $^{d}$ | 21.74 ± 0.34 $^{d}$ |
| **C18:1 n-9** | 22.94 ± 0.85 | 22.96 ± 0.88 $^{a}$ | 30.65 ± 1.86 $^{d}$ | 21.42 ± 0.58 $^{a}$ | 18.73 ± 0.66 $^{c}$ | 21.12 ± 0.46 $^{a}$ |
| **C18:2 n-6** | 8.83 ± 0.41 | 10.98 ± 0.81 $^{b}$ | 15.28 ± 0.54 $^{d}$ | 7.84 ± 0.23 $^{a}$ | 8.58 ± 0.16 $^{a}$ | 12.67 ± 0.32 |
| **C18:3 n-3** | 1.26 ± 0.02 | 0.84 ± 0.03 | 1.75 ± 0.06 $^{c}$ | 2.07 ± 0.02 $^{d}$ | 1.65 ± 0.06 $^{b}$ | 1.72 ± 0.08 $^{b}$ |

When groups that were given $H_2O_2$ and the extract are compared to the $H_2O_2$ group, it was observed that compared to the $H_2O_2$ group, palmitic acid amounts demonstrated a partial increase difference S + $H_2O_2$ group relative to the $H_2O_2$ group ($P < 0.05$) (TABLE III). Compared to the control group, on the other hand, it was determined that palmitic acid levels demonstrated partial decreases in the $H_2O_2$, B + $H_2O_2$, C + $H_2O_2$, and D + $H_2O_2$ groups ($P < 0.05$, $P > 0.05$) (TABLES II and III). Palmitoleic acid levels, on the other hand, demonstrated a decrease in the B, S, $H_2O_2$, B + $H_2O_2$, C + $H_2O_2$ relative to the control group ($P < 0.001$, $P < 0.01$). Groups that were given $H_2O_2$ and tomato extracts, when compared to the $H_2O_2$ group, once again demonstrated that fatty acid decreased in the B + $H_2O_2$ group ($P < 0.05$), while demonstrating increases in the C + $H_2O_2$, D + $H_2O_2$, and S + $H_2O_2$ groups ($P < 0.05$, $p < 0.01$) (TABLES II, III).

It was found that stearic acid levels, when compared to the control group, demonstrated increases in varying levels in both the groups made only with the addition of tomato and all groups which were given tomatoes + $H_2O_2$ ($P < 0.05$, $P < 0.01$, $P < 0.001$). Even though oleic acid amounts demonstrated distinct increases in the B group relative to the control group ($P < 0.001$), it was determined that significant decreases in the D group were observed ($P < 0.01$). When the same fatty acid in the groups with added $H_2O_2$ and tomato extract is compared to the $H_2O_2$ group, partial decreases were determined in the C + $H_2O_2$, D + $H_2O_2$, and S + $H_2O_2$ groups ($P < 0.05$) (TABLE III). The linoleic acid level, relative to the control group, was found to demonstrate distinct increases in the B and S groups ($P < 0.001$). When a comparison between the same fatty acid in groups with added $H_2O_2$ and extract with the H group is conducted, significant increases were observed in the B + $H_2O_2$ group. It was determined that linolenic acid, which is among essential oil acids (C18:3, n-3), demonstrated varying levels of increases relative to the control group in groups that were given extract + $H_2O_2$ ($P < 0.05$, $P < 0.01$, $P < 0.001$). Myristic and lauric acids, relative to the control group, were observed to decrease in varying levels in both the group with added extract and the group with added extract + $H_2O_2$ (TABLES II and III).

**TABLE III**
**The variation of fatty acid percentage in *S. cerevisiae* pellets supplemented with tomato (*Solanum lycopersicum L.*) extracts and $H_2O_2$ compared to the $H_2O_2$ group**

| Fatty acids | Experimentation Groups | | | | |
|---|---|---|---|---|---|
| | $H_2O_2$ | B+ $H_2O_2$ | C+ $H_2O_2$ | D+ $H_2O_2$ | S+ $H_2O_2$ |
| **C16**:0 | 25.77 ± 0.76 $^{b}$ | 25.54 ± 0.24 $^{b, a}$ | 26.92 ± 0.39$^{a, a}$ | 26.47 ± 0.55 $^{b, a}$ | 28.87 ± 0.31 $^{a, b}$ |
| **C16:1 n**-7 | 15.13 ± 0.24 $^{c}$ | 13.78 ± 0.17 $^{d, b}$ | 17.05 ± 0.35 $^{b, b}$ | 18.34 ± 0.32 $^{a, c}$ | 18.28 ± 0.44 $^{a, c}$ |
| **C17**:0 | 0.55 ± 0.03 | 0.54 ± 0.02 $^{a}$ | 0.65 ± 0.07 $^{a}$ | 0.69 ± 0.04 $^{a}$ | 0.87 ± 0.08 $^{a}$ |
| **C17**:1 | 0.44 ± 0.04 | 0.43 ± 0.02 $^{a}$ | 0.47 ± 0.03 $^{a}$ | 0.51 ± 0.02 $^{a}$ | 0.48 ± 0.02 $^{a}$ |
| **C18**:0 | 21.30 ± 0.51$^{d}$ | 20.83 ± 0.67 $^{c,a}$ | 22.43 ± 0.89 $^{d,a}$ | 21.07 ± 0.74 $^{d,a}$ | 21.51 ± 0.84 $^{d,a}$ |
| **C18:1 n-9** | 22.96 ± 0.88 $^{a}$ | 22.69 ± 0.86 $^{c,a}$ | 19.06 ± 0.78 $^{d,b}$ | 19.21 ± 0.86 $^{d,b}$ | 18.88 ± 0.56 $^{d,b}$ |
| **C18:2 n-6** | 10.98 ± 0.81 $^{b}$ | 13.26 ± 0.74 $^{d,c}$ | 10.18 ± 0.63 $^{b, a}$ | 10.87 ± 0.78 $^{b, a}$ | 7.73 ± 0.82 $^{a, c}$ |
| **C18:3 n**-3 | 0.84 ± 0.03 | 1.84 ± 0.04 $^{c}$ | 1.88 ± 0.06 $^{d}$ | 1.46 ± 0.07 $^{b}$ | 1.78 ± 0.09 $^{b}$ |

Studies conducted until today have reported that $H_2O_2$ is not freely diffused in cell membranes, and that with the *E. coli, S. cerevisiae* and the mammal cell being in the forefront, the Jurcat-T cells form a barrier system against $H_2O_2$ diffusion [34]. It was determined that fatty acid synthetase (FAS) is a key molecule in making $H_2O_2$'s entrance into the cell more difficult through the forming of a related mechanism [35].

In another study regarding this mechanism, the effect of nitrogen sources on the fatty acid makeup of *S. cerevisiae* was inspected and the fact that the yeast environment impacted the fatty acid makeup was emphasized. As a reason for this finding, the fact that the plasma membrane is the first cell part that contacts the environment the yeast is contained in [36]. Another similar study conducted fatty acid analysis through 4 different methods and 2 separate *S. cerevisiae* strands. And at the end of this study, it was determined that the method involving the mixture also containing $CCl_4$ demonstrated more distinct increases compared to other methods [37].

This study, which used tomato extracts, showed similar results to the previous study in the reference. Specifically, it was determined that relatively to the group with added tomato extracts, the $H_2O_2$ group demonstrated generally higher fatty acid contents. In the study conducted with tomatoes, it was determined that the C12:0, C14:0, C18:1 n-9 values and the $H_2O_2$ group's Σ MUFA values were found to be higher than the $H_2O_2$ + D groups.

Additionally, increases in C18:1 n-9, C18:2 n-6 and C18:3 n-3 fatty acids were detected in the tomato-added groups (B, B1, B2, B3, B4, B + $H_2O_2$, B2 + $H_2O_2$ and B3 + $H_2O_2$). This suggests that carbon sources such as fruit sugars and other minerals found in different fruit extracts in the growing medium of *Saccharomyces cerevisiae* cause an increase in fatty acid levels and radical groups in the fruit extracts.

This is because the varying proportions of these substances and carbon sources found in fruits can affect the activity of enzymes responsible for the synthesis of certain fatty acids, leading to changes in the fatty acid composition. On the other hand, as a cause for this, it was reported that *S. cerevisiae*'s exposure to $H_2O_2$ may lead to differences in the permeability and biophysiological properties of lipid components [34].

### Lipophilic molecule analysis

As lipophilic molecules, the analysis of ADEK vitamins and phytosterols are depicted in tables 4 and 5. The vitamin K2 amount , relative to the control group, was observably at high levels in groups with added tomato extracts ($P < 0.05$, $P < 0.01$) while out of the groups with simultaneously added tomato extracts and $H_2O_2$, the C + $H_2O_2$, D + $H_2O_2$, and S + $H_2O_2$ groups demonstrated it at high levels ($P < 0.05$, $P < 0.01$), δ-tocopherol levels, compared to the control, demonstrated decreases in the B, C, B + $H_2O_2$, and C + $H_2O_2$ groups while ($P < 0.05$, $P < 0.01$) demonstrating no statistical differences in other groups.

When the vitamin D levels are compared relative to the control group, the C, D, and S groups demonstrated high levels of the vitamin ($P < 0.05$, $P < 0.01$). $H_2O_2$ and extract-added groups, relative to the $H_2O_2$ group, demonstrated a leaning to increases in the B + $H_2O_2$, C + $H_2O_2$, D + $H_2O_2$, and S + $H_2O_2$ groups ($P < 0.05$, $P < 0.01$). The α-Tocopherol content, on the other hand, demonstrated decreases in the B, D, and S groups ($P < 0.01$, $P < 0.05$) while demonstrating a partial increase in the C group ($P < 0.05$) (TABLE IV).

**TABLE IV**
**The variation of lipophilic molecules in *S. cerevisiae* pellets supplemented with tomato (*Solanum lycopersicum L.*) extracts compared to the control group (µg/g)**

| Lipophilic molecules | Experimentation Groups | | | | | |
|---|---|---|---|---|---|---|
| | Control | $H_2O_2$ | B | C | D | S |
| **K2** | 0.41 ± 0.02 | 0.59 ± 0.05 $^{b}$ | 0.64 ± 0.03 $^{b}$ | 0.57 ± 0.04 $^{b}$ | 0.75 ± 0.06 $^{b}$ | 0.82 ± 0.08 $^{c}$ |
| **δ -Tokoferol** | 0.28 ± 0.01 | 0.22 ± 0.02 $^{a}$ | 0.13 ± 0.01 $^{c}$ | 0.19 ± 0.02 $^{b}$ | 0.23 ± 0.02 $^{a}$ | 0.24 ± 0.02 $^{a}$ |
| **D2** | 1.06 ± 0.02 | 0.61 ± 0.08 $^{c}$ | 1.02 ± 0.03 $^{a}$ | 1.57 ± 0.12 $^{b}$ | 1.97 ± 0.09 $^{c}$ | 1.39 ± 0.07 $^{b}$ |
| **α-Tokoferol** | 2.61 ± 0.13 | 0.58 ± 0.03 $^{d}$ | 1.59 ± 0.11 $^{c}$ | 3.37 ± 0.17 $^{b}$ | 1.88 ± 0,16 $^{c}$ | 2.29 ± 0.18 $^{b}$ |
| **Ergosterol** | 121.52 ± 2.21 | 111.33 ± 4.07 $^{a}$ | 78.35 ± 1.52 $^{c}$ | 126.57 ± 1.29 $^{a}$ | 113.54 ± 1.46 $^{a}$ | 134.82 ± 2.3 $^{b}$ |
| **K1** | 0.68 ± 0.07 | 1.47 ± 0.07 $^{d}$ | 2.94 ± 0.12 $^{d}$ | 2.96 ± 0.13 $^{d}$ | 2.18 ± 0.11 $^{d}$ | 2.62 ± 0.14 $^{d}$ |
| **Stigmasterol** | 21.48 ± 0.97 | 11.47 ± 0.05 $^{d}$ | 10.82 ± 0.87 $^{c}$ | 20.16 ± 0.32 $^{a}$ | 22.38 ± 0.88 $^{a}$ | 21.11 ± 0.67 $^{a}$ |
| **β-Sitosterol** | 1.04 ± 0.05 | 1.31 ± 0.05 $^{b}$ | 3.88 ± 0.28 $^{d}$ | 3.17 ± 0.11 $^{d}$ | 1.93 ± 0.12 $^{c}$ | 3.23 ± 0.29 $^{d}$ |

The α-Tocopherol levels were found to increase in all groups with added extract. ($P < 0.001$). However, groups with added $H_2O_2$, when compared to the control group, demonstrated low levels ($P < 0.01$) (TABLE V). Moreover, $H_2O_2$ and extract-added groups were determined to demonstrate distinctly higher levels of K1 compared to the control group levels ($P < 0.001$) (TABLE V). Even though the ergosterol level in the membrane structure of yeast cells was found to demonstrate significant differences in the B group relative to the control group ($P < 0.01$), it was found to partially increase in the S group ($P < 0.05$). From the $H_2O_2$ added groups, B + $H_2O_2$ demonstrated distinct decreases ($P < 0.01$), D + $H_2O_2$ demonstrated distinct increases ($P < 0.01$). Stigmasterol levels, relative to the control group, were found to decrease in the B group ($P < 0.001$). $H_2O_2$-added groups, when compared, demonstrated that partial increases occurred in the C + $H_2O_2$ and D + $H_2O_2$ groups relative to the $H_2O_2$ group.

However, if the control group and the $H_2O_2$ group are compared, it was found that decreases of varying levels occurred relative to the control group ($P < 0.05$, $P < 0.01$, $P < 0.001$) (Table V). From phytosterols, β-Sitosterol levels were found to demonstrate increases of varying levels in both the groups with added extract and added extract + $H_2O_2$ ($P < 0.05$, $P < 0.01$, $P < 0.001$) (TABLE IV, V).

**TABLE V**
**The variation of lipophilic molecules in *S. cerevisiae* pellets supplemented with tomato (*Solanum lycopersicum L.*) extracts and $H_2O_2$ compared to the $H_2O_2$ group (µg/g)**

| Lipophilic molecules | Experimentation Groups | | | | |
|---|---|---|---|---|---|
| | $H_2O_2$ | B+ $H_2O_2$ | C+ $H_2O_2$ | D+ $H_2O_2$ | S+ $H_2O_2$ |
| **K2** | 0.59 ± 0.05 | 0.49 ± 0.03 $^{b}$ | 0.64 ± 0.05 $^{b}$ | 0.68 ± 0.06 $^{b}$ | 0.63 ± 0.07 $^{c}$ |
| **δ-Tokoferol** | 0.22 ± 0.02 | 0.12 ± 0.02 $^{c}$ | 0.18 ± 0.03 $^{a}$ | 0.24 ± 0.04 $^{a}$ | 0.28 ± 0.05 $^{a}$ |
| **D2** | 0.61 ± 0.08 | 0.85 ± 0.07 $^{b}$ | 0.95 ± 0.08 $^{b}$ | 1.96 ± 0.07 $^{c}$ | 1.46 ± 0.08 $^{b}$ |
| **α-Tokoferol** | 0.58 ± 0.03 | 1.56 ± 0.08$^{d}$ | 1.81 ± 0.11 $^{d}$ | 2.08 ± 0.05 $^{d}$ | 1.63 ± 0.05$^{d}$ |
| **Ergosterol** | 111.33 ± 4.07 | 90.18 ± 3.03 $^{c}$ | 101.83 ± 2.45 $^{a}$ | 149.56 ± 2.12 $^{c}$ | 115.71 ± 2.30 $^{a}$ |
| **K1** | 1.47 ± 0.07 | 1.42 ± 0.12 $^{a}$ | 1.56 ± 0.13 $^{a}$ | 1.36 ± 0.11 $^{a}$ | 1.46 ± 0.14 $^{a}$ |
| **Stigmasterol** | 11.47 ± 0.05 | 11.77 ± 0.11 $^{a}$ | 17.04 ± 0.22 $^{b}$ | 23.24 ± 0.28 $^{b}$ | 12.49 ± 0.77 $^{a}$ |
| **β-Sitosterol** | 1.31 ± 0.05 | 2.27 ± 0.11 $^{b}$ | 1.81 ± 0.06 $^{b}$ | 3.37 ± 0.21 $^{d}$ | 2.33 ± 0.11 $^{b}$ |

The pigment that provides many antioxidant properties to tomatoes as well as its red color is lycopene, being a pigment known to be capable of preventing degenerative illnesses such as cancer and can fight against these illnesses [38]. Lycopene is more effective in catching single oxygens than other carotenoids. The active radicals of carotenoids, by connecting direct radicals, electron transfer, or by giving $H_2$, have an oxidative; been reported that this can deactivate materials [32]. Some conducted studies have reported that β-carotenes, which have high oxidative properties (due to their chain breaking capabilities), lose their antioxidant activities if oxygen pressure increases [39].

Moreover, carotenoid consumption, has been reported to decrease the occurrence rate of embolism, bone calcification, and nerve illnesses as it increases [40]. Outside of β-carotene, tomatoes also contain carotenoids such as phytoene, phytofluene, phenolic compounds such as coumaric and chlorogenic acids, high levels of vitamin C, and tocopherol materials, increasing the nutritiousness of tomatoes.

On the other hand, studies conducted on tomatoes when inspected, reveal that the number of studies aiming to understand the impact of the phytochemicals in tomatoes on health is low [41]. A study conducted, the vitamin synthesis of different sugars (glucose, molasses, saccharose, and lactose) in isolated *Rhodotolura glutinis* yeast was studied. At the conclusion of the study, it was reported that the highest vitamin synthesis occurred in environments with high saccharose, while the same impact was not observed in environments with lactose [41].

This study aimed to investigate the effects of tomato extracts on vitamin and tocopherol content in yeast, thereby pioneering research into the role of these mechanisms in human metabolism. As a result of this study, groups with added tomatoes, relatively to the control group, demonstrated increases in K1, K2 vitamins β-Sitosterol, vitamin C contents in the B, C, D, S groups while the α-Tocopherol content increased in the C group, and the Ergosterol content demonstrated increases in the C and S groups in the studies conducted relative to the $H_2O_2$ group, we also determined that β-Sitosterol contents increased in the B + $H_2O_2$, C + $H_2O_2$, D + $H_2O_2$, S + $H_2O_2$ groups, Ergosterol content increased in the D + $H_2O_2$ and S + $H_2O_2$ groups.

Likely, these differences suggest that they are due to the sugar content of different tomato batches. This study has shown that these changes are parallel to the rate of change in lipophilic molecules in the fruit extracts used. Alongside the benefits of carotenoid and vitamin contents, studies reporting on the fact that phenolic components in tomatoes are effective in preventing allergic, inflammatory, microbial and cancer illnesses, moreover that it is effective in preventing microbial and cancer illnesses and preventing the forming of illnesses such as strokes and heart attacks are also present. All these properties formed the basis of this study.

## CONCLUSION

In light of the data we obtained, we determined that *S. cerevisiae* developed a defense mechanism against $H_2O_2$ radicals in the groups where tomato (*Solanum lycopersicum* L.) extracts were added to the nutrient medium. In our results, the increase in GSH values, especially in tomato groups added with $H_2O_2$, showed the protective effect of tomatoes against oxidative stress of $H_2O_2$.

The same can be said for lipophilic molecules. However, this study observed slight differences in antioxidant activity and the key compounds affecting it, namely lipophilic molecules and fatty acid content, in samples collected from different regions. In light of the findings of this study, it can be said that in order for these compounds in tomatoes to contribute to human nutrition as food additives, it is necessary to isolate these compounds from tomatoes (*Solanum lycopersicum* L.), conduct studies with other yeast species, and perform animal experiments if necessary.

At the same time, if these beneficial compounds are isolated, they may hold promise for the production of supplements and drugs for the prevention and treatment of cancer and similar diseases.

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**Data availability statement**

The data that support the findings of this study are available from the corresponding author, [SA], upon reasonable request.

**Conflicts of Interest**

The authors declare no conflict of interest.

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