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Responses of *Saccharomyces cerevisiae* Cells Grown in Cultures Prepared from Different Tea Infusions to Oxidative Stress

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ABSTRACT

Tea is one of the most consumed beverages. *Saccharomyces cerevisiae*, a model organism in studies on oxidative stress and toxicology, was used to investigate the effects of tea on oxidative stress induced by H_2O_2. *S. cerevisiae* cultures were prepared from black, green and white tea infusions and incubated at 30°C for 72 hours. Glutathione-S-transferase enzyme activity and total protein spectrophotometric, malondialdehyde, glutathione and alpha-tocopherol and ergosterol analyses from cell pellets obtained from cultures were performed by HPLC, and fatty acids were performed by GC device. Although protein level in tea infusion groups was higher ($p<0.001$) compared to control and H_2O_2 groups, malondialdehyde level decreased ($p<0.001$). Glutathione and GST levels were decreased in other tea infusion groups except for black tea infusion and black tea infusion+H_2O_2 groups ($p<0.001$). Ergosterol levels decreased in both tea infusion and H_2O_2+tea infusion groups ($p<0.05$; $p<0.001$). While palmitic acid increased ($p<0.01$) in tea infusions and H_2O_2 groups, palmitoleic acid decreased ($p<0.05$). Stearic and oleic acid levels decreased in tea infusion groups ($p<0.05$). As a result, it has been observed that the water-soluble components of tea have effects on fatty acid biosynthesis, other metabolic products and oxidative stress.

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Farklı Çay İnfüzyonlarından Hazırlanan Kültürlerde Yetiştirilen *Saccharomyces cerevisiae* Hücrelerinin Oksidatif Strese Tepkileri

ÖZET

Çay, en çok tüketilen içeceklerden biridir. Çayın, H_2O_2 ile oluşturulan oksidatif stres üzerindeki etkilerini araştırmak için *Saccharomyces cerevisiae*, oksidatif stres ve toksikoloji ile ilgili çalışmalarda model organizma, kullanılmıştır. Siyah, yeşil ve beyaz çay infüzyonlarından *S. cerevisiae* kültürleri hazırlandı ve 30°C'de 72 saat inkübe edildi. Kültürlerden elde edilen hücre peletlerinden glutatyon-S-transferaz enzim aktivitesi ve total protein spektrofotometrik, malondialdehit, glutatyon ve alfa tokoferol ve ergosterol analizleri HPLC ile, yağ asitleri ise GC cihazı ile yapıldı. Çay infüzyon gruplarında protein düzeyi kontrol ve H_2O_2 gruplarına göre daha yüksek ($p<0.001$) olmasına rağmen, malondialdehit düzeyi azalmıştır ($p<0.001$). Siyah çay infüzyonu ve siyah çay infüzyonu+H_2O_2 grupları dışındaki diğer çay infüzyon gruplarında glutatyon ve GST seviyeleri azaldı ($p<0.001$). Ergosterol seviyeleri hem çay infüzyonu hem de H_2O_2+çay infüzyonu gruplarında azaldı ($p<0.05$; $p<0.001$). Çay infüzyonlarında ve H_2O_2 gruplarında palmitik asit artarken ($p<0.01$), palmitoleik asit azalmıştır ($p<0.05$). Çay infüzyon gruplarında stearik ve oleik asit seviyeleri azaldı ($p<0.05$). Sonuç olarak, çayın suda çözünen bileşenlerinin, yağ asidi biyosentezi, diğer metabolik ürünlerin ve oksidatif stres üzerinde etkileri olduğu görülmüştür.

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INTRODUCTION

Camelia sinensis L. is a flowering plant species belonging to the Theaceae family, and it has been stated that tea obtained from its leaves and buds has a history dating back to 5000 years and approximately 2/3 of the world's population consumes tea (Elhadad et al. 2020; Takim & Aydemir 2022). Moreover, it has been reported that the antioxidant effect of tea is due to the flavonoids it contains (Jain et al. 2013). White tea is obtained from the buds of the tea plant and subjected to the withering process (Hilal & Engelhardt 2009). Green tea is a fermented tea produced by drying fresh leaves and exposing them to evaporation. It has a complex composition consisting of 15-20% protein by dry weight, 5-7% soluble carbohydrate components, 5% minerals and 1-4% amino acid mixture (Cabrera et al. 2006). Black tea is produced as a fermented tea following the withering of the leaves and becomes palatable by being completely oxidized during processing (Negishi et al. 2004).

The phenolic substances with the highest antioxidant effect in tea leaves are catechins, which constitute 30% of the dry weight (Almajano et al. 2008; Horžić et al. 2009). Theaflavins and thearubigins in black tea and the catechins in green tea are the components responsible for the physiological effects of tea. It has been emphasized that epigallocatechin-3-gallate in green tea is generally the most biologically active compound (Lorenz 2013). Phenolic compounds demonstrate antioxidant effects by binding free radicals, forming chelates with metals, and inactivating the lipoxygenase enzyme (Huang et al. 2005). Nevertheless, the polyphenolic compounds in tea have beneficial antioxidant activity, they also have pro-oxidant properties due to their molecular properties (Babich et al. 2008; Lorenz 2013).

Reactive Oxygen Species (ROS), which are also formed under normal metabolic conditions, can change the cellular redox balance with the increase of oxidative stress caused by the effect of toxic substances (Meng et al. 2017). Hydrogen peroxide (H_2O_2) formed during metabolic activities causes the formation of hydroxyl (OH•), a reactive and harmful free oxygen radical.

S. cerevisiae is preferred as a model organism for toxicological studies and to identify new natural compounds with antioxidant functions to protect against oxidative stress. Thence, due to the metabolic similarity of *S. cerevisiae* cells to humans, the reactive effects of infusions of tea, which is widely consumed by humans, on *S. cerevisiae* were investigated. Since tea varieties are produced by subjecting the leaves to different processes, it is important to know which tea is a potentially more beneficial beverage in terms of antioxidant activity. Thus, it was aimed to investigate the biochemical effects on *S. cerevisiae* cells in in vitro cultures with H_2O_2 added by preparing 0.2% infusions from white, green and black tea species.

MATERIALS and METHODS

Preparation of Tea Infusions and In Vitro Yeast Cell Culture

After brewing 2 g of dry tea leaves in 1 L of distilled water at room temperature for 12 hours, tea infusions were prepared by filtering them through filter papers.

Groups within the experimental study: Control (C), H_2O_2 (H), Black tea (BT), Green tea (GT), White tea (WT), Black tea + H_2O_2 (BTH), Green tea + H_2O_2 (GTH), and White tea + H_2O_2 (WTH) groups were separated.

For each group, 20 g peptone, 20 g yeast extract, 40 g D (+) Glucose were weighed and dissolved in 1000 ml distilled water. Each culture medium was divided into 200 ml cap bottles (n=5) and then sterilized in an autoclave at 121°C for 15 minutes. After the cell medium was cooled, yeast cells were inoculated into the culture medium of all groups under sterile conditions. In addition, 100 µL of 37% H_2O_2 was added to the H, BTH, GTH and WTH groups. All groups were incubated at 30°C for 72 hours. Cell pellets were then collected by centrifugation at 6000 rpm at +4°C for 5 minutes and their wet weights were determined. Cell pellets were washed with isotonic physiological buffer solution and cleared of culture broth residues.

Extraction of Cell Pellets with Tris-ETDA Buffer

Cell pellets were homogenized in 20 mM Tris-EDTA (pH=7.4) buffer at +4°C and then centrifuged. Supernatants were collected after centrifugation for analysis of total protein, GSH, GSSG, MDA, and Glutathione S-transferase (GST). The remaining cell pellet was homogenized with a 3/2, (v/v) n-hexane/isopropanol mixture (Hara & Radin 1978) and the supernatant fraction was used for fatty acid, D2, alpha-tocopherol and ergosterol analysis (Katsanidis & Addis 1999).

Measurement of GSH and GSSG by HPLC

2 ml of supernatant was taken and centrifuged by adding 2 ml of 10% perchloric acid. Then, 1 ml of supernatant was taken and analyzed by HPLC device (Prominence LC-2030 C 3D plus, Shimadzu, Kyoto, Japan). 50 mM $NaClO_4$ was used as mobile phase (acidified with 0.1% H_3PO_4) and ODS-3 column was used as analytical column. Measurements were made at a wavelength of 215 nm (Yilmaz et al. 2009).

MDA Assay by HPLC

2 ml of Tris-EDTA homogenate was taken and 2 ml of 10% perchloric acid was added and centrifuged. Then, 1 ml of supernatant was taken and analyzed by HPLC device (Prominence LC-2030 C 3D Plus, Shimadzu, Kyoto, Japan). A mixture of 30 mmol KH_2PO_4 and methyl alcohol (82.5-17.5%, pH=4) was used as mobile phase and ODS-3 column was used as analytical column. Measurements were made at a wavelength of 254 nm (Karatepe 2004).

Analysis of Fatty Acids

For extraction of lipids, cell pellets were homogenized with a 3/2, (v/v) n-hexane/isopropyl alcohol mixture and centrifuged (Hara & Radin 1978). For fatty acid analysis, 2 ml of supernatant was taken and 2% methanolic H_2SO_4 was added. It was left for 12 hours at 55°C. Fatty acid methyl esters were extracted with n-hexane and analyzed in GC 2010 Plus gas chromatography (Shimadzu, Kyoto Japan). Rxt-2330 GC column (Supelco, Sigma, USA) was used as the column during the analysis. For analysis, the column temperature was 138-218°C, the injection temperature was 240°C, and the detector temperature was 290 was programmed. Helium gas was used as the carrier gas. The results were determined as %. Calculation was done with the LC/GC 5.91 operating program.

Analysis of Lipophilic Molecules

10% KOH was added to the supernatants obtained for analysis and mixed and kept at 85°C for 15 minutes. Unsaponified lipophilic molecules were extracted with 2x5 ml hexane and evaporated under nitrogen gas. The remaining residue was dissolved in 1 ml of mobile phase mixture acetonitrile/methanol (75+25%, v/v). The mobile phase flow rate was 1 ml min^{-1}. The PDA detector was used in the analysis and Nucleodur C18 (Germany) was used as the column. Analyzes were performed at 205 nm wavelength (Katsanidis & Addis 1999; Lopez-Cervantes et al. 2006).

Determination of GST Activity

Glutathione S-transferase activity was performed according to the method of Habig et al. (1974). 1 mM 1-chloro-2,4-dinitrobenzene, 1 mM GSH and 0.1 M phosphate buffer (pH=6.5) were used for this measurement. The reaction mixture was completed to 2.9 ml with phosphate buffer by adding 0.1 ml of GSH and 0.1 ml of CDNB, and the reaction was started by adding 0.1 ml of cell homogenate. Next, the change in absorbance was measured at a wavelength of 340 nm. Results were calculated using the GST standard.

Measurement of Total Protein

The amount of protein in the samples was measured spectrophotometrically according to the method described by Lowry et al. (1951).

Statistical Analysis

SPSS package program was used for statistical analysis of the results. ANOVA and LSD test were used to determine the differences between the groups. Statistical significance was accepted as $p<0.05$. Data are given as mean± Standard error of mean .

RESULTS

Changes in total protein levels in cell pellets treated with tea infusions and hydrogen peroxide are shown in Table 1. Total protein levels in the tea infusion groups were approximately twice that of the C and H groups. The amount of MDA was found to be higher in all infusion groups compared to the control group. However, when the H group added hydrogen peroxide was compared with the BTH and GTH groups, a decrease was observed in both groups ($p<0.05$, $p<0.01$). Although there was a partial decrease in the WTH group, no statistical difference was found.-

Table 1. Variation of total protein, MDA, GSH, GSSG, and GST concentrations in tea infusions and hydrogen peroxide added cell pellets (g cell pellet)

Çizelge 1. Çay infüzyonlarında ve hidrojen peroksit eklenmiş hücre peletlerinde (g hücre peleti) toplam protein, MDA, GSH, GSSG ve GST konsantrasyonlarının değişimi

Groups	Total Protein (mg g^{-1})	MDA (nmol g^{-1})	GSH (µg g^{-1})	GSSG (µg g^{-1})	GST (µg g^{-1})
C	1.81±0.15	13.62±0.46	220.16±3.00	26.99±1.81	19.88±0.59
H	1.63±0.07 ^{a}	28.54±0.98 ^{d}	152.29±4.44 ^{d}	35.42±1.55 ^{d}	23.80±0.60 ^{b}
BT	3.62±0.07 ^{d}	20.09±1.11 ^{d}	310.48±4.89 ^{d}	58.32±1.17 ^{d}	28.00±0.89 ^{d}
BTH	3.52±0.32 ^{d}	24.79±0.76 ^{d}	251.65±6.05 ^{c}	56.77±1.91 ^{d}	23.03±1.67 ^{b}
GT	3.35±0.33 ^{d}	25.99±1.03 ^{d}	98.24±1.26 ^{d}	20.29±0.57 ^{c}	11.43±0.30 ^{c}
GTH	2.62±0.16 ^{c}	22.77±0.89 ^{d}	105.07±3.95 ^{d}	20.03±0.58 ^{c}	9.10±0.24 ^{d}
WT	3.49±0.20 ^{d}	17.26±0.98 ^{b}	181.33±4.87 ^{c}	19.41±0.70 ^{c}	16.63±0.58 ^{b}
WTH	3.77±0.13 ^{d}	27.31±0.64 ^{d}	98.78±4.63 ^{d}	8.10±0.70 ^{d}	14.60±1.36 ^{b}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2

** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.

a: $p>0.05$ is not statistically significant; b: $p<0.05$ is statistically significant; c: $p<0.01$ is more statistically significant; d: $p<0.001$ is most statistically significant.

The GSH level was decreased in the H group compared to the control group. It was higher in the BT and BTH groups than in the control group. In addition, a significant decrease was observed in GSH levels in GT, GTH, WT and WTH groups compared to both C and H groups ($p<0.05$). GSH and GSSG levels were significantly decreased in GT, GTH, WT and WTH groups compared to C and H groups ($p<0.05$).

Glutathione S-Transferase (GST, EC.2.5.1.18) activity were higher in H, BT and BTH groups than in group C ($p<0.05$). However, the activity of GST decreased in GT, GTH, WT and WTH groups (Table 1).

The α-tocopherol level did not change in the BT group compared to C, but decreased in the GT and WT groups and in the tea infusion groups with added H_2O_2 ($p<0.001$). In the tea infusion and tea infusion groups to which H_2O_2 was added, the ergosterol level decreased at different rates compared to the control group ($p<0.05$). Although vitamin D level was higher in the BT group, it decreased in the GT and WT groups ($p<0.05$) (Table 2).

Table 2. Concentrations of lipophilic molecules in cell pallets developed in the medium of tea infusions (μg g^{-1} cell pellet)

Çizelge 2. Çay infüzyonları ortamında geliştirilen hücre paletlerindeki lipofilik moleküllerin konsantrasyonları (μg g^{-1} hücre peleti)

Groups	α-Tocopherol	Vitamin D2	Ergosterol
C	2.64±0.38	1.14±0.12	165.45±7.63
H	3.78±0.34 ^{c}	0.90±0.13 ^{a}	151.21±6.09 ^{b}
BT	2.78±0.40 ^{a}	2.65±0.94 ^{b}	101.94±7.02 ^{c}
BTH	1.00±0.12 ^{d}	1.04±0.41 ^{a}	90.69±5.82 ^{c}
GT	1.16±0.32 ^{d}	0.79±0.15 ^{b}	79.76±11.12 ^{d}
GTH	1.11±0.22 ^{d}	1.04±0.41 ^{a}	73.34±6.93 ^{d}
WT	0.73±0.47 ^{d}	0.73±0.47 ^{b}	116.51±31.68 ^{b}
WTH	1.26±0.27 ^{c}	0.91±0.12 ^{a}	122.82±6.43 ^{b}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2
** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.
a: $p>0.05$ is not statistically significant; b: $p<0.05$ is statistically significant; c: $p<0.01$ is more statistically significant; d: $p<0.001$ is most statistically significant.

The amount of palmitic acid (16:0) was found to be significantly increased in all tea infusion groups compared to the control group ($p<0.05$). In addition, the 16:0 level was found to be high in the tea infusion groups with added hydrogen peroxide. However, palmitoleic acid (16:1, n-7) levels were decreased at different rates in both BT, GT, and WT groups and in tea infusion groups with added hydrogen peroxide compared to the control group ($p<0.05$). The amounts of stearic acid (18:0) and oleic acid (18:1, n-9) in the tea infusion groups decreased at different rates compared to the control group.

It was observed that 18:0 and 18:1 n-9 levels were decreased in the hydrogen peroxide and tea infusion groups compared to the control group ($p<0.05$), while there was no difference at the 18:0 level in the GTH group ($p>0.05$). The amount of 18:1, n-7 was higher in GT, WT, GTH, and WTH groups compared to the control group ($p<0.05$). While the amount of heptadecanoic acid (17:0) decreased in GT and WT groups compared to the control group ($p<0.05$), the amount of cis-heptadecenoic acid (17:1) increased in the tea infusion groups ($p<0.001$, $p<0.05$). The increase in the amount of 17:1 was observed to increase only in the WTH group among the groups to which hydrogen peroxide was added ($p<0.001$). (Table 3-4). The amount of cis-heptadecenoic acid (17:1) was found to be significantly higher in the tea infusion groups and in the WTH group compared to the control group (Table 3-4).

Although the amount of linoleic acid (18:2, n-6) decreased in the tea infusion groups, the amount of linolenic acid (18:3, n-3) was lower in the GT group and higher in the BT and WT groups ($p<0.001$) (Table 3). While the amount of 18:2 decreased in H and WTH groups ($p<0.05$), no difference was found in BTH and GTH groups ($p>0.05$) (Table 4). Lignoceric acid (24:0) was significantly higher in the GT group ($p<0.01$) and partially decreased in the WT group ($p<0.05$). Yet, the amount of 24:0 was slightly higher in the BTH group ($p<0.05$), and no difference was found in the other groups (Table 3-4). Moreover, the total saturated fatty acid ratio increased in the tea infusion groups, BTH and GTH groups compared to the control group, it was determined that the total unsaturated fatty acid ratio decreased (Table 3-4).

DISCUSSION

When the control and tea infusion groups were compared, the protein levels in the infusion groups were found to be higher than the control group (Table 1). In the analyzes made, it was stated that more than 1% of the dry weight of tea contains free amino acids.

It has been determined that these amino acids consist of 20 L-form amino acids that take part in protein synthesis. (Tan et al. 2011). Free amino acids in the structure of tea are active components that affect the taste, aroma and color of tea (Pastoriza et al. 2017). The reason why the amount of protein in the groups of tea infusion is higher than that of the C and H groups may be due to the high content of free amino acids in the infusions of the teas.

Table 3. Comparison of fatty acid ratios with control, hydrogen peroxide, and tea infusions added groups (%)
Çizelge 3. Yağ asidi oranlarının kontrol, hidrojen peroksit ve çay infüzyonları eklenmiş gruplarla karşılaştırılması (%)

Fatty acids	C	BT	GT	WT
10:0	0.84±0.06	1.57±0.10 ^{d}	1.36±0.22 ^{d}	0.33±0.01 ^{d}
12:0	2.97±0.09	4.04±0.24 ^{c}	4.17±0.32 ^{c}	2.49±0.07 ^{a}
14:0	2.74±0.20	4.61±0.40 ^{c}	4.34±0.09 ^{c}	5.32±0.11^{d}
16:0	29.80±0.50	33.53±0.33 ^{c}	33.87±0.74 ^{c}	37.64±0.43 ^{d}
16:1, n-7	26.57±0.65	24.70±0.43 ^{b}	25.59±0.68 ^{b}	15.23±0.32 ^{d}
17,0	0.62±0.03	0.46±0.05 ^{a}	0.35±0.02 ^{c}	0.34±0.05 ^{c}
17:1	0.51±0.05	2.36±0.17 ^{d}	3.01±0.11 ^{d}	0.85±0.85 ^{b}
18:0	10.69±0.40	7.48±0.55 ^{c}	8.99±0.38 ^{b}	8.03±0.17 ^{b}
18:1, n-9	18.11±0.55	13.38±0.66 ^{c}	9.33±0.23 ^{d}	17.86±0.22 ^{a}
18:1, n-7	1.69±0.09	2.11±0.13 ^{a}	4.44±0.93 ^{d}	7.95±0.83 ^{d}
18:2, n-6	3.22±0.32	2.12±0.22 ^{b}	2.13±0.15 ^{b}	1.98±0.10 ^{b}
18:3, n-3	1.31±0.15	2.40±0.21 ^{d}	0.70±0.12^{d}	3.38±1.02 ^{d}
24:0	0.99±0.36	1.23±0.20 ^{a}	1.72±0.50 ^{c}	0.60±0.09 ^{b}
∑ Saturated	48.62±1.59	52.93±1.29 ^{b}	54.80±1.89 ^{c}	53.75±1.11 ^{c}
∑ Unsaturated	51.32±1.72	47.07±1.69 ^{b}	45.20±1.32 ^{c}	46.25±2.09 ^{b}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2
** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.
a: p>0.05 is not statistically significant; b: p<0.05 is statistically significant; c: p<0.01 is more statistically significant; d: p<0.001 is most statistically significant.

Table 4. Comparison of fatty acid ratios with control, hydrogen peroxide, and tea infusions added groups (%)
Çizelge 4. Yağ asidi oranlarının kontrol, hidrojen peroksit ve çay infüzyonları eklenmiş gruplarla karşılaştırılması (%)

Fatty acids	H	BTH	GTH	WTH
10:0	0.84±0.05 ^{a}	1.33±0.03 ^{d}	0.96±0.02 ^{a}	0.31±0.01 ^{d}
12:0	3.38±0.23 ^{a}	3.93±0.23 ^{b}	3.27±0.13 ^{a}	2.35±0.14 ^{a}
14:0	2.91±0.11 ^{a}	2.13±0.13 ^{a}	3.10±0.28 ^{a}	3.06±0.22 ^{a}
16:0	30.46±0.61 ^{a}	34.53±0.78 ^{c}	32.53±0.35 ^{b}	35.56±0.46 ^{c}
16:1,n-7	29.99±0.58 ^{c}	23.08±1.09 ^{b}	22.38±0.14 ^{b}	18.86±0.33 ^{c}
17,0	0.53±0.02 ^{a}	0.46±0.05 ^{a}	0.34±0.09 ^{b}	0.33±0.05 ^{b}
17:1	0.61±0.04 ^{a}	0.59±0.07 ^{a}	0.78±0.06 ^{a}	1.59±0.13 ^{d}
18:0	8.37±0.31 ^{c}	9.17±0.22 ^{a}	11.31±0.47 ^{a}	6.88±0.26 ^{c}
18:1, n-9	18.07±0.49 ^{a}	17.15±0.33 ^{a}	15.41±0.42 ^{b}	16.86±0.60 ^{b}
18:1, n-7	1.40±0.06 ^{a}	2.09±0.20 ^{a}	2.81±0.28 ^{b}	8.29±0.28 ^{d}
18:2, n-6	2.24±0.19 ^{b}	2.81±0.15 ^{a}	3.84±0.18 ^{a}	2.54±0.31 ^{b}
18:3, n-3	0.55±0.19 ^{d}	0.79±0.14 ^{c}	1.90±0.18 ^{b}	2.83±0.44 ^{d}
24:0	0.65±0.04 ^{a}	1.74±0.14 ^{b}	1.37±0.35 ^{a}	0.54±0.17 ^{a}
∑ Saturated	47.14±1.37 ^{a}	53.32±1.58 ^{c}	52.88±1.69 ^{c}	49.03±1.31 ^{a}
∑ Unsaturated	52.86±1.43 ^{a}	46.68 ±1.71 ^{c}	47.12 ±0.98 ^{c}	50.97±1.75 ^{a}

C: Control; H: H_2O_2; BT: Black Tea; BTH: Black Tea+H_2O_2; GT: Green Tea; GTH: Green Tea+H_2O_2; WT: White Tea; WTH: White Tea+H_2O_2
** The evaluations in the tables were made between the control group and other groups, and the statistical signs are between the control group and other groups.
a: p>0.05 is not statistically significant; b: p<0.05 is statistically significant; c: p<0.01 is more statistically significant; d: p<0.001 is most statistically significant.

Lipid peroxidation level (MDA) was higher in the H group and in the tea infusion groups to which H_2O_2 was added. However, the MDA level in the tea infusion groups was lower than in the H group. These results show that the water-soluble components in the tea content reduce the formation of free radicals in cell metabolism. According to Kırmızıkaya et al. (2021), in

their study with tea types, stated that the MDA level of red meats exposed to tea infusion at +4°C for a week decreased compared to the control group.

However, in association with higher MDA levels in the yeast groups developed in tea infusions than in the control group, it can be concluded that these compounds are due to the pro-oxidant effects. Tang et al. (2019) stated that there are components such as polyphenols, alkaloids, pigments and saponins in the extracts of tea plants. Therefore, the presence of more than one component in a mixture and not knowing their amounts may cause pro-oxidant effects and may cause harmful results instead of beneficial effects.

Yen et al. (1997) reported that tea extracts may act as pro-oxidants or antioxidants due to their ability to reduce iron and scavenge oxy-radicals. Tang et al. (2019) suggested in their study that although the extracts of some tea varieties contain very high levels of phytochemicals, their bioavailability is low. On the other hand, Dani et al. (2008) emphasized that the addition of polyphenols to the commonly used cell culture medium can cause significant amounts of hydrogen peroxide formation and OH• radical formation and oxidative stress.

GSH is among the most important molecules of the protective system against free radicals in all cells. GSH level was decreased in group H compared to group C. It was higher in BT and BTH groups than in group C. However, a decrease was observed in other tea groups. GSH is an antioxidant molecule that is mainly effective against hydrogen peroxide. A decrease in GSH can be seen naturally in groups to which hydrogen peroxide is added. Because when there is not enough NADPH molecule in the environment, the GSSG level also rises. We think that this hypothesis is supported by the higher GSSG level compared to group C.

The GSH level was found to be higher in the BT and BTH groups than in the C group. We think that this is due to the high levels of free amino acids such as glutamic acid, cysteine and glycine (Tang et al. 2019) used in GSH synthesis in black tea infusions. Because black tea is prepared by crushing wilted tea leaves, it contains limited amounts of catechin and more abundant theaflavin (Babich et al. 2008). We think that this view can be supported by the decrease in GSH in the BTH group compared to the BT group. Because HO• radicals formed from hydrogen peroxide convert the H_2O_2 of the GSH molecule to water and molecular oxygen to prevent radical formation, while the formation of GSSG molecules in the cell increases.

The high amount of GSSG in the BT and BTH groups may be due to insufficient glutathione reductase enzyme activity or the lack of sufficient NADPH molecules. In addition, high GSH levels in yeast are an important criterion for recovery from acute peroxide stress (Spector et al. 2001). It was observed that both GSH and GSSG molecules decreased significantly in green and white tea infusion groups compared to C and H groups. We think that the reason for the decrease in GSH and GSSG here is partially different from other groups. Tea plants contain different polyphenols (Almajano et al. 2008). Many of these polyphenols can have a pro-oxidant effect on GSH synthesis and lead to a decrease in GSH level.

Although the majority of research on black tea and health focuses on its antioxidant properties, the catechins and theaflavins found in green tea have also been reported to exhibit pro-oxidant behavior (Babich et al. 2008). When the total amount of GSH decreases, the rate of GSSG may decrease. The high MDA level and low GSH and GSSG in the WTH group supports this hypothesis. Another reason is that all physiological events in the cell system work at a normal level and there is no need for high-level synthesis of molecules such as GSH.

Glutathione S-Transferases (GSTs, EC.2.5.1.18) are a superfamily of multifunctional enzymes that detoxify xenobiotic compounds by binding GSH to a hydrophobic substrate. According to results, activity of GST was higher in H, BT and BTH groups compared to the control group. The yeast cell would need to synthesize the GST enzyme under reasonable conditions to protect itself against toxic H_2O_2 in the environment. GST is an antioxidant enzyme that has a purifying effect on both the toxic effect of H2O2 and the cellular damage caused by the hydroxyl radical. This may be due to the high activity of GST in group H. The activity of GST was higher in the BT group. This result is in parallel with the increase in the activity of GSH. Similarly, the decrease in the activity of GST in green and white tea groups is parallel to the decrease in the amount of GSH. Black tea contains limited amounts of catechins and larger amounts of theaflavin derivatives, which are considered biologically important and health-beneficial active ingredients (Leung et al. 2001). The differences between black tea and green and white tea may be due to the further processing of black tea. As is known, some of the compounds with pro-oxidant effect may be lost during processing.

The *S. cerevisiae* cell has the capacity to synthesize all the molecules it needs in the current culture medium. It also synthesizes lipophilic molecules such as ergosterol and α-tocopherol. As shown in Table 2, α-tocopherol level decreased in GT and WT groups. In addition, it decreased in tea groups with H_2O_2 added compared to C and H groups.

Alpha-tocopherol is a molecule with a very high antioxidant potential, tends to interrupt free radical chain reactions and protect polyunsaturated fatty acids and cell membranes (Tufarelli 2014; Izah et al. 2019). We think that the reason for the increase in α-tocopherol levels in the H_2O_2 added groups and the

decrease in the tea added groups is the prooxidant effect of the water-soluble molecules in the tea infusions on the synthesis. Because when the cell encounters abnormal situations, it activates its mechanisms and protects itself. An example of this is the increase in the amount of α-tocopherol in the H group. However, although the MDA level was high in the groups to which tea infusion was added, the lack of high synthesis of molecules such as α-tocopherol that disrupt the free radical reaction supports the above hypothesis.

Ergosterol is one of the most important lipophilic molecules in the membrane structure of yeast cells, and it plays an important role in vitality, membrane fluidity, and permeability (Hata et al. 2010). *S. cerevisiae* contains all genes necessary for the production of ergosterol (Mantzouridou et al. 2009). Ergosterol, one of the most important sterols in cells such as *S. cerevisiae*, is used as a precursor of vitamin D2. Ergosterol levels decreased in the groups in which both tea infusion and tea infusion and H_2O_2 were added compared to the control group.

Under stress conditions, it has also been found that the ability of the yeast cell to tolerate stress is closely related to ergosterol levels. It has been stated that the ergosterol content of yeast resistant to low sugar fermentation conditions is high and *S. cerevisiae*, which is exposed to alcohol effect for a long time, can increase the ergosterol content in the cell membrane to protect the membrane structure. Similar results were obtained in the *S.cerevisiae* erg6 mutant, in which the ergosterol content in the cell membrane decreased and the cells became more susceptible to alcohol stress (Hu et al. 2017).

As shown in Table 3, the amount of palmitic acid was found to be significantly increased in the whole tea infusion and H_2O_2 added tea infusion groups compared to the control group. These results lead us to the conclusion that some water-soluble compounds in tea plants have a positive effect on fatty acid synthetase enzyme activity and increase the transcription of this enzyme. Fatty acid biosynthesis in yeast cells is similar to higher organisms, although there are some differences. Malonyl-CoA molecules synthesized by the Acetyl-CoA Carboxylase enzyme serve as the precursor molecule for palmitic acid synthesis formed by the multifunctional fatty acid synthetases in yeast, consisting of two subunits encoded by FAS1 and FAS2. The end product of fatty acid synthetases is palmitic acid and can be converted to stearic acid in yeast with an extension encoded by ELO1. Both palmitic and stearic acids can be converted to the monounsaturated fatty acids palmitoleic and oleic acids in yeast by the enzyme Steroyl CoA desaturase (SCD) encoded by the OLE1 gene.

S.cerevisiae can de novo form all essential fatty acids and also readily absorbs a wide variety of exogenous long-chain saturated and polyunsaturated fatty acids from the growth medium and rapidly incorporates them into membrane lipids. As seen in Table 3, while palmitic acid level increased in *S.cerevisiae* groups developed in tea groups compared to control group, palmitoleic acid level decreased. It has been reported that the membrane lipids of *S.cerevisiae* may be affected by different physiological and nutritional conditions. It has been suggested that OLE1 gene expression, which provides the synthesis of single-double-bonded unsaturated fatty acids, is affected by factors such as carbon source, nutritive fatty acids, metal ions, and oxygen levels, and may respond differently to these (Martin et al. 2007).

It was observed that both 18:0 and 18:1 in the fatty acid composition decreased at different rates compared to the control group (Table 3). Although 18:0 decreased in all groups developed in hydrogen peroxide and tea infusion, it was determined that it decreased 18:1 only in GTH and WTH groups (Table 4). It can be thought that the fluctuations in the 18:0 and 18:1 levels are due to the changes in the activities of the elongaz and SCD enzymes.

Ding et al. (2015) found that some molecules in tea inhibit the expression of genes that play a key role in lipid biosynthesis and the biosynthesis of the SCD enzyme. In addition, Yuan et al. (2009) stated that SCD reduced mRNA expression in adipose tissue of rats. Ding et al. (2015) found that the amount of 16:0 increased partially and the amount of 18:0 significantly increased in the groups given PTE. They emphasized that the ratio of unsaturated fatty acids such as 18:1 and the ratio of 18:1n-9/18:0 decreased with increasing PTE concentration. Ding et al. (2015) were found to be similar to the results of their study.

Heptadecenoic acid (17:1 n-9) was found over 1% in BT and GT groups. It was also found to be higher in the WT group than in the control group (Table 3). The amount of 17:1 in the WTH group, one of the tea infusion groups to which hydrogen peroxide was added, was determined as 1.59% (Table 4). We think that this increase in the amount of 17:1, which is generally more than 1% in fatty acid composition, may be due to the effect of water-soluble compounds found in aqueous infusions of tea species. It has been reported that when propionic acid is present in the normal culture medium of *S.cerevisiae* cells, this fatty acid facilitates heptadecenoic acid synthesis by increasing the activity of the propionyl-CoA synthetase enzyme (Pronk et al. 1994). The 17:1 level of *S.cerevisiae* cells developed in the white tea infusion was found to be higher than the C and H groups. This may be due to the high amount of organic acids, acetic acid and propionic acid found in white tea. Dias et al. (2013) reported that the main components of tea are proteins, polysaccharides, polyphenols, minerals and trace elements, amino and organic acids, lignins and

methyl xanthines. When the chemical composition of kombucha infusions was examined, significant levels of propionic acid were found (Huang et al. 2016).

CONCLUSION

Due to the metabolic similarity of *S. cerevisiae* cells to humans, the reactive effects of commonly consumed tea on *S. cerevisiae* in the oxidative stress environment created by hydrogen peroxide were investigated. Though it is known that teas inhibit lipid peroxidation, the present study has shown that infusion forms of teas have pro-oxidant properties. However, it has also been found to have effects on fatty acid synthesis, vitamin synthesis, and the synthesis and use of glutathione, the cell's defense molecule. Although this study was conducted on *S. cerevisiae*, the present results showed that widely consumed tea varieties should be consumed more carefully by humans due to their health effects. More comprehensive and molecular studies are needed to define the specific effects of teas on the metabolism of organisms.

Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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