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INVESTIGATION OF THE ANTIOXIDANT EFFECT OF TWO THIOLS, γ-GLUTAMYL CYSTEINE AND GLUTATHIONE, IN SUNFLOWER OIL UNDER ACCELERATED STORAGE

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ABSTRACT

Sunflower oil is an oil that is prone to oxidation due to its chemical structure and prevention of such oxidation is widely studied. This study assessed the the effectiveness of γ-glutamyl cystein (γGC, GC) and glutathione (GSH), in preventing oxidation of sunflower oil stored at 50°C for a period of 15 days. TBHQ was used as a positive control while no additive oil selected as a negatif control. Oxidation level indicators such as peroxide (*PV*), free fatty acidity (*FFA*), p-anisidine (*p-AV*) but also total oxidation (*Totox*), colour (*L**, *a**, *b**) and fatty acid profile were determined. At the end of storage, oxidation in sunflower oil was substantially reduced by 40 mg/L of GC. Analysis with 2,2-diphenyl-1-picrylhydrazyl (DPPH) resulted in the following order of IC_{50}; T (0.08±0.01), BHA (0.13±0.03), GC (0.3±0.01), GSH (0.41±0.00), BHT (0.42±0.02). The samples resistance to the generation of primary and secondary oxidation products was T>GCT>GC>GSHT>GSH>C for up to 15 days under storage conditions. The fatty acid profile analysed by GC/MS further demonstrated that these thiols outperformed the control group in terms of performance. Findings demonstrated that GC, precursor of GSH, has stronger antioxidant activity than GSH. As a result, it is recommended to be explored as a potential source of antioxidants in applications for the food industry to prevent lipid oxidation.

1.Introduction

Based on its high content of unsaturated fatty acids, especially essential 9-cis and 12-cis-octadecadienoic acid, sunflower oil is considered as one of the highest quality vegetable oils for human nutrition (Mariamenatu & Abdu, 2021). Beside this, it is also one of the most prone to oxidation upon storage (Lu et al., 2022). Compounds formed by lipid oxidation such as hydroxyperoxides, aldehydes, carbonyl compounds, hydrocarbons (alkane, alkene) formed during oil oxidation have adverse affects on human health but also alter the chemical and sensory properties of oils. Oxygen is mainly responsible of the initiation and acceleration of oils oxidation (Echegaray et al., 2022) although, temperature, light, degree of unsaturation and the presence of metal ions such as copper and iron also accelerate oxidation (Bastos, 2010). Therefore, it is challenging to avoid oil oxidation without adding any antioxidant substances.

Antioxidants are compounds that prevent or delay oxidative reactions based by a combination of scavenging free radicals, chelating prooxidative metals, quenching singlet

oxygen among other (Choe & Min, 2009). Based of their lower cost and greater efficiency, antioxidants obtained by chemical synthesis, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary hydroquinone (TBHQ) are commonly utilized in the food industry (Abdelazim et al., 2013). However, they are also associated with issue in safety to use (Honold et al., 2016). Consumers avoid the use of these products due to the fact that these chemically synthesized antioxidants have a harmful affect on human health. Therefore, the demand for natural antioxidants is constantly increasing.

In cells, different thiol compounds has antioxidants fuction prevent damage caused by free radicals and provide detoxification. Glutathione (GSH), cysteine (CYS), homocysteine (HCYS), N-acetylcysteine (NAC), captopril (CAP) and γ-glutamylcysteine (γGC,GC) are the best known biological thiols (Ulrich & Jacob, 2019). GC, a dipeptide composed of cysteine and glutamate is the cellular precursor of glutathione. GC and GSH are thought to protect cells against oxygen toxicity by destroying peroxides, disulfides, and other oxygen-generated species (Kritzinger et al., 2013). In particular, GSH is used in the cosmetic, medical and food industries as an active ingredient of these products to alleviate harmful oxidative processes and prevent the formation of toxic compounds such as radicals (superoxide, hydroxyl, peroxyl, alkoxyl) and non-radicals (hydrogen peroxide hydroperoxide etc.). It is also used to strengthen and repair skin whitening due to its anti-aging affect (Hamad et al., 2018).

The oxidative stability of oils can be determined during storage and packaging under normal ambient conditions. However, oxidation takes a long time to occur, which makes it impractical for routine analysis. Therefore, accelerated oxidation test have been developped, notably by increasing storage temperature to 50 °C upon 15 days (Bandyopadhyay, 2008).

The objectives of this study were to evaluate the ability of GSH and its precursor GC to prevent or at least reduce oxidation of sunflower oil and to compare thier antioxidant capacity between them and other used industrial antioxidants. For this purpose, sunflower oil was subjected to accelerated oxidation under laboratory conditions and the affect of temperature, sun light and oxidation was monitored during storage period.

2.Material and methods

2.1. Materials and reagents

The refined organic sunflower oil used in this study was purchased from Beyorganic company located in Istanbul, Turkey. Glutathione (GSH), γ-glutamylcystein (GC), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich Co. (St. Louis, MO, USA).

2.2. Oxidation of Sunflower oil in the presence of glutathione, γ-glutamyl cysteine and TBHQ

Accelerated oxidation test were performed according to the method of Papadopoulou and Roussis (Papadopoulou et al., 2008). Briefly, 50 ml of sunflower oil were stored in 100 ml flasks in the presence of glutathione (GSH; 40 mg/L), γ-glutamylcystein (GC; 40 mg/L), TBHQ (T; 200 mg/L), γ-glutamylcystein+TBHQ (GCT; 40 mg/L+40 mg/L) and glutathione+TBHQ (GSHT; 40 mg/L+40 mg/L). Oil without additives was selected as a control (C; no additive). The experiment carried out with 3 repetition for each sample. Prior incubation, antioxidants were dispersed in the oil phase in an ultrasonic water bath (Bandelin, RK 1028H, Berlin) for 10 minutes. All samples were incubated at 50°C and in sun light conditions for 15 days. Analyzes were performed in 2 replications on days 0, 3, 6, 9, 12 and 15.

2.3. Determination of free radical scavenging activity for glutathione, γ-glutamyl cysteine, BHA, TBHQ, BHT

The radical scavenging activity of GSH and GC and commercial antioxidants used in food, namely BHA, BHT, TBHQ were tested according to Blois et al (1958) with some modification (Blois, 1958). Briefly, solutions of

GSH, GC, BHA, TBHQ and BHT were prepared in ethanol at concentrations ranging from 0.04 to 1.5 mM. 390 µl of DPPH solution (25 mg/L ethanol) was mixed to 100 µl of the different antioxidant solutions. After, 30 minutes of incubation in the dark at room temperature (25°C). The results was measured at 517 nm absorbance by visible spectrophotometer (Perkin- Elmer, USA). For the reference sample, a mixture of ethanol and DPPH was used (Kamkar et al., 2010). The scavenger activity was calculated according to Eq. (1):

DPPH radical scavenging activity (%) = [(Ab-As) / Ab] * 100 (1)

Ab is the blank absorbance and As is the absorbance of the sample.
IC_{50} was calculated for each sample on inhibition %.

2.4. Determination of peroxide value, free fatty acids and p-Anisidine value

To determine the peroxyde value (*PV)*, 1 g of sunflower oil was dissovled by gentle mixing in 25 mL of chloroform-acetic acid solution (2:3 v/v) mixture. One ml of saturated potassium iodide solution was then added and the mixture was incubated in the dark for 5 minutes. It was then diluted by adding 75 mL of distilled water. One mL of 1% starch solution was added and the final solution was titrated with 0.01N sodium thiosulfate ($Na_2S_2O_3$). The *PV* values were calculated according to AOCS, 2003, cd 8-53 method (Matthäus 2006) using Eq. (2):

PV= (V×N×1000 meq g O_2/kg) /m (2)

V: Spent sodium thiosulfate solution, mL
N: Normality of sodium thiosulfate solution
m: sample weight, g.

The amount of free fatty acids (*FFA*) in oil samples was calculated as oleic acid. To determine the *FFA*, 1g of oil sample was dissolved in ethanol-diethyl ether (1:1 v/v) solution. The resulting solution was then titrated by 0.1 N KOH in ethanol in the presence of phenolphthalein. Fatty acidity content was calculated according to the AOCS Ca 5a-40 method (Tyburczy et al., 2013) using Eq. (3):

% *FFA* = V x N x Ma /m (3)

V: 0.1 N spent ethanolic potassium hydroxide solution, mL
N: Normality of ethanolic potassium hydroxide solution,
Ma: Molecular weight of oleic acid,
m: Sample weight, g.

The p-anisidine value (*P-Av*) was determined according to the modified AOCS 1990 method. First 0.5 g of oil sample was dissolved in 10 ml of hexane was measured at a wavelength of 350 nm with Lambda 25 UV-VIS (Perkin- Elmer, USA) spectrophotometer. Then 2.5 ml of the solution was taken and 0.5 ml of p-anisidine solution (0.25 g/100 ml of acetic acid) was added, and absorbance was measured at 350 nm (Pocklington & Dieffenbacher, 1988). Finally, the *P-Av* was calculated according to Eq. (4):

P-Av= 10*(1.2 x A2- A1)/m (4)

A2: last reading,
A1: first reading,
m: sample quantity.

2.5. Measurement of Colour (L*, a*, b*) and calculation of total oxidation (Totox) value

Colour measurement in oil was performed by Chroma meter CR-400(Conica Minolta, Japan) colourimeter. In the CIELAB colour space *L**, *a**, and *b** values indicate lightness, green to red, and blue to yellow, respectively. The *Totox* value in oil was calculated on *PV* and *P-Av* according to theEq. (5):

Totox =(2*PV) + p-AV (5)

2.6. GC-MS analysis of Fatty Acids

For determination of fatty acid composition, the methylesters were prepared by cold transmethylation with potassium hydroxide according to IUPAC 2.301-2.302 method (Paquot, 2013). Briefly, 10 mg oil was dissolved in 10 ml hexane solvent. Then 0.5 ml of 2N

KOH was added to the oil sample and left in the dark for 2 hours. Then, the upper phase was collected and filtered with a 0.45 µm filter before being analysed by gas chromatography-mass spectrometry (Agilent- 7890B GC -7010B MS) with an autosampler (Gerstel, Germany) equipped with the flame ionization detector (FID). A capillary DB-WAX column (Agilent J&W; 60m x 0.25 µm x 0.25µm) was used. The oven temperature was held at 50ºC for 1 min, raised to 200ºC at a rate of 25°C/min held for 10 min and then to 230ºC at a rate of 3 ºC/min held this temperature for 25 min. The injector (250 ºC) and detector temperatures (300ºC) were set. The sample size was 1µl and the the flow rate of helium carrier gas was 1 mL/min. The split used was 1:40. The identification of fatty acid was determined by checking with the retention times of known fatty acid standards and given as a percentage of the total.

2.7. Statistical analysis

The results of the analyzes were evaluated by analysis of variance (ANOVA), and Tukey tests. "Rstudio 2022.02.03 version "agricolae" package was used for statistical analysis. Principle component analysis was done by Xlstate 2023 software. Results were evaluated in biplot.

3.Results and Discussions

3.1 Antioxidant properties

Figure 1 presents the antioxidant properties using DPPH assay of the GC and GSH, along with the reference standards TBHQ, BHA, and BHT. DPPH is a stable free radical widely used for screening compounds with free radical scavenging abilities (Sethi et al., 2020).

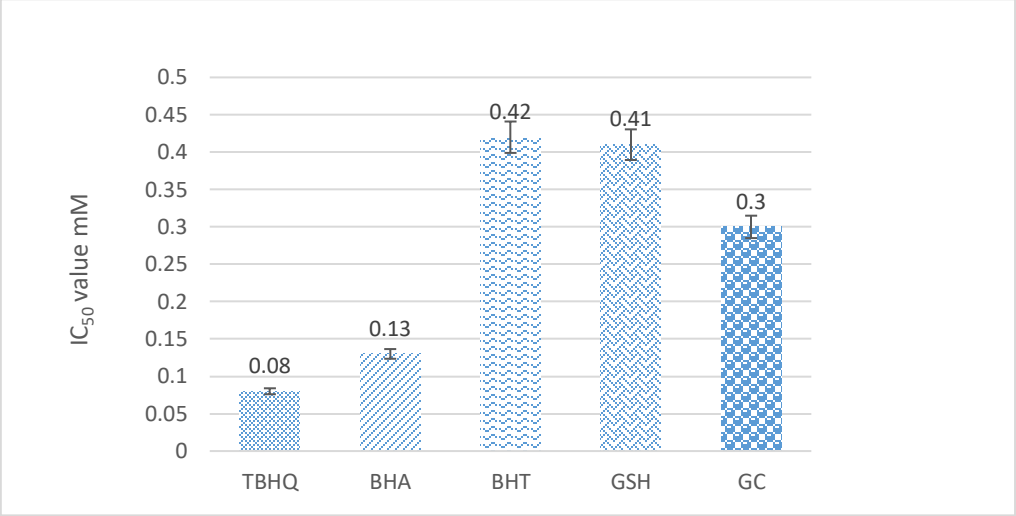

Figure 1. DPPH radical-scavenging activity of GC and GSH compared with BHA, BHT, and TBHQ at different concentrations. Mean ± SD, *n*=3.

In the present study, IC_{50} values were found in the range of 0.08 to 0.42 mM. It was observed that the DPPH radical-scavenging activity of the GC and GSH were found to be higher than the TBHQ and BHA, but lower than the BHT (Figure 1). Their scavenging activity of DPPH radicals decreased in the following line TBHQ>BHA>GC>GSH>BHT. Superoxide anion and hydroxyl groups are two of the most important free radicals. Superoxide anions are produced by adding an electron to molecular oxygen and are harmful reactive oxygen species because they damage cellular components in biological systems (Yu et al.,2016). It was noted that the presence of -SH groups in the medium significantly eliminated the superoxide anion and hydroxyl groups. Since GC and GSHS contain -SH groups, it has a powerful scavenging affect on free radicals (Jie et al.,2016).

Many studies regarding DPPH radical-scavenging activity in the literature are on plant extracts and oils comparing with synthetic antioxidants. Although there are not many studies on DPPH scavenging activity of GC and GSH in oil, Fileger et al (2020) compared hydroalcoholic extracts from *Aegopodium podagraria* L. with ascorbic acid and glutathione. It was stated that the inhibition concentration of glutathione was around 65% for 0.7 mM. In present study, it was found 64.2 % for 0.75mM glutathione. In another study, the synergistic effect of glutathione (50-200 μM) with various flavones was studied using DPPH. It was noted that inhibition % increased when GSH amount raised (Pereira et al., 2013).

TBHQ IC_{50} value was determined as 6.87 μg/ml and 29.81 μg/ml in two studies (Zheng et al., 2011;Gharib et al., 2013). In the present study, the IC_{50} value corresponds to 13μg/ml (0.08 mM) for TBHQ. Chen et al (2014) compared rosemery extract with commercial antioxidants in their study, the IC_{50} value was ordered as TBHQ>BHA>BHT. Our finding was also in agreement with the results reported by previous studies (Laulloo et al., 2015; Prevc et al., 2013). The maximum limit of commercial antioxidants usage is determined as 200 mg/L by the Food Drug Administration (FDA). Although the determined IC_{50} values of these antioxidants are low, the limit used is high considering their negative effects on human health (Sharma et al., 2019).

3.2. Determination of *PV* and *FFA* and *P-Av* in accelerated oxidation of sunflower oil

The degree of oxidation in oils is usually characterised by the peroxide value that relates to hydroperoxides, the primary oxidation products that are unstable and readily decompose to form mixtures of mainly volatile aldehyde compounds. It is known that oils are rapidly oxidized by heat, light and air, thus increasing the *PV* (Gharby et al., 2011). The affect of antioxidants on peroxide value in the sunflower oil samples is in shown in Figure 2. Results show that *PV* increases linearly for all samples with storage time. The increase in PV accelerated after 3rd day. As a result, the control sample had the highest PV till the end of storage time 4.67– 168.33 meq O2/kg. When the samples are compared in terms of PV, the order was C>GSH>GC>GSHT>GCT>T. There are significant differences between groups, particularly at the end of oxidation ($p<0.05$). As mentioned above, GC, a dipeptide, is the precursor of GSH. According to the DPPH result, GC, which showed higher activity than GSH, showed a stronger antioxidant affect against GSH during accelerated oxidation. Crapiste et al (1999) measured the peroxide value of sunflower oil before and after storage. The values obtanied indicated that while the peroxide value was 3.36 meq/kg at the beginning, it reached 90 meq/kg in 30 days at 47°C. These results are in line with the peroxide value (168.33 meq/kg, at 50°C, 15 days) recorded in the present study corraborating the impact of temperature. In this study, we aimed to highlight any synergistic affects of GC and GSH with TBHQ, as antioxidant in sunflower oil that will allow subsequent utilisation of lower concentration of TBHQ. According to the results, values close to each other were GCT, 19.33 meq O2/kg and GSHT, 27.33 meq O2/kg ($p<0.05$), TBHQ was recorded when 200 mg was used (15 meq O2/kg ($p<0.05$)). This means that it showed a positive synergistic affect with the synthetic antioxidant. Glutathione was compared with synthetic antioxidant, and its antioxidant affect was investigated by measuring the peroxide value in butter. Glutathione affect showed results close to BHA at the end of storage (Papadopoulou et al., 2008). There are also studies close to our results to prevent the increase of PV in sunflower oil (Erol et al., 2022; Naserzadeh et al., 2018; Saeed et al., 2022). *FFA* refers to the free fatty acids and is expressed in mg of potassium hydroxide or sodium hydroxide required to neutralize 1 gram of fat. *FFA* is an important quality index for oil and is constantly used as a shelf-life monitoring parameter in oil. An increase in *FFA* means a decrease in stability to oxidation. This is one of the important indicators that the oil will start to become rancid (Frankel, 2014). The concentration of *FFA* calculated as % oleic acid are shown in Figure 2**.**

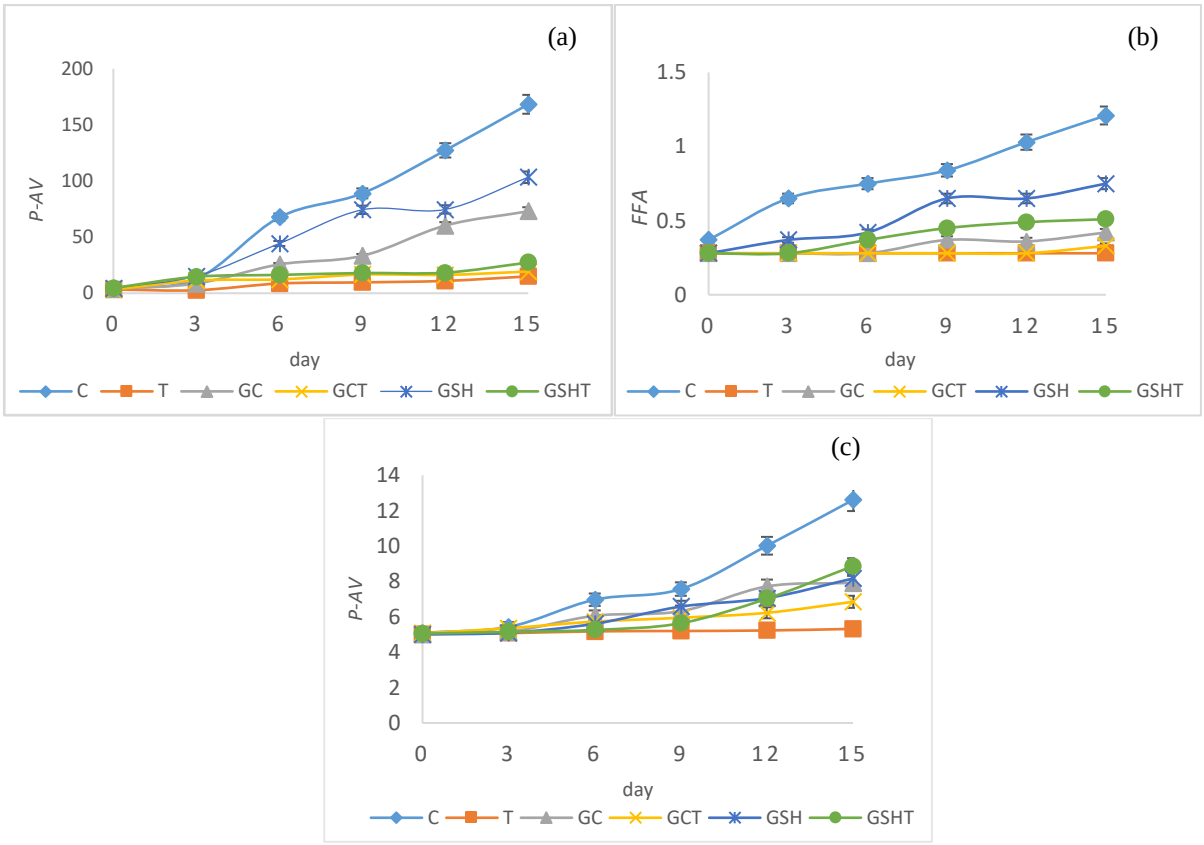

Figure 2. Affect of adding TBHQ, GC, and GSH on peroxide (a), FFA (b) and p-anisidine value (c) in sunflower oil under accelerated oxidation conditions. C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3.

According to the *FFA* data, the best result during the storage period belongs to the oil sample containing TBHQ. A significant difference was not observed between groups till 1the 6th day ($p<0.05$). At the end of storage time, *FFA* of sunflower oil sample C, TBHQ, GC, GCT, GSH, GSHT were 1.21±0.16, 0.28±0.00, 0.42±0.00, 0.33±0.08, 0.75±0.16, 0.51±0.08 (% oleic acid) respectively. According to Duncan's multiple comparison, there was no significant difference between T, GC, GCT, but there was a statistical difference between these groups with GSH and GSHT. This shows that GC has antioxidant affects close to TBHQ. In addition, when we examined the combination of GC and GSH with TBHQ, GCT showed the highest antioxidant result. Generally *FFA* result increased in parallel with *PV* results (Dhibi et al., 2022). *FFA* measurement by the accelerated oxidation method in sunflower oil has been measured in many studies. While *FFA* TBHQ value (0.28 oleic acid %) in this study had a similar to the literature (Chen et al., 2013), GCT results showed higher antioxidant activity than the literature compared to plant-derived antioxidants. GCT results in this study showed higher antioxidant activity than literature (Chang et al., 2015; Iqbal et al., 2007; Mei et al., 2014).

The oxidative degradation of oils begins with the formation of primary compounds such as hydroperoxides. They react to undesirable secondary oxidation products, such as aldehydes, alkanes, esters, alcohols etc. Secondary oxidation products are determined by *P-Av* in oils and fats (Ramadan & Mörsel, 2004). *P-Av* results are shown in Figure 2. The *P-Av* results were parallel to the *PV* results and increased on the 3rd day, with the highest value at the end of storage.

The data presented in Figure 2 showed that sample C has the highest secondary oxidation products. A statistically significant difference was observed in *P-Av* between the GC and GSH at the end of storage. After 15 days the increase in *P-Av* value is in the order of C (7.95±0.68) > GC (6.37±0.65) > GSH (6.26±0.55) > GSHT (6.17±1.14) > GCT (5.87±0.66) when all days are averaged. As a result of *P-Av*, lower value was recorded in all the doped groups than the control group. Although GC and GSH values were close, there was a statistically significant difference between TBHQ and GSH groups, but no difference was observed between TBHQ and GC groups ($p<0.05$). There is no difference between GC and GCT. It is thought that if the GC concentration is increased in oil, it will slow down the increase of *P-Av* without TBHQ.

Two major methods seperate the oxidation level; the peroxide number measures the hydroperoxide level, while the anisidine value measures the secondary oxidation level. In a similar study the ability of glutathione and N-acetyl-cysteine for all conditions showed results close to the BHA sample in *P-Av* measurement. As a result N-acetyl-cysteine and GSH may be taken into account as antioxidants in corn oil during storage, cooking or frying (Papadopoulou et al., 2008).

When the two measurements are made together and applied to the formula, the *Totox* level is revealed and the oxidation level is determined. According to many studies, the maximum levels are 5 meq/kg for peroxide level, 20 for anisidine and 26 for totox (FAO, 2015). *Totox* value results shown in Figure 3.

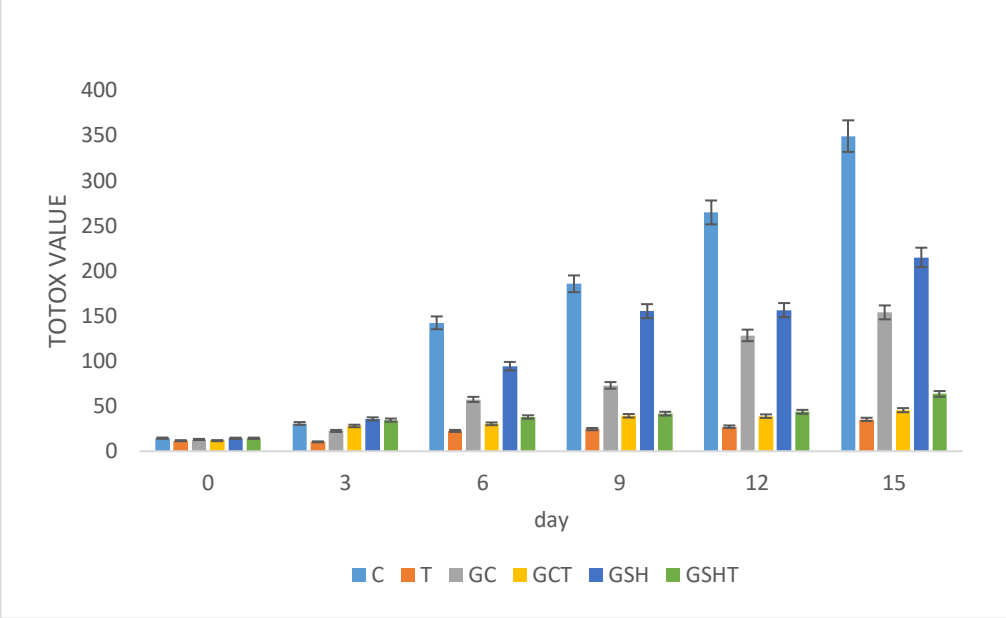

Figure 3. Comparison of p-Anisidine value of different treatments of sunflower oil during 15 days at 50˚C. C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3

At the end of the storage period, the *totox* value of all groups was above 26 meq O_2/kg. However, when the antioxidant groups were compared with TBHQ protected oil, a rapid increase was observed in the total oxidation value at the end of the 3rd day in parallel with the *PV* and *P-Av*. In the end of storage time *totox* value of C, T, GC, GCT, GSH, GSHT determined (349.28±6.79) meq O_2/kg, (35.32±1.22) meq O_2/kg, (153.92±4.71) meq O_2/kg, (45.52±3.99) meq O_2/kg, (214.84±2.34) meq O_2/kg, (63.54±0.56) meq O_2/kg respectively. In a study in which lemon peel was used as an antioxidant, the *totox* value was compared with the oil sample containing BHT and the *totox* value was found higher than in the study compared to data given by (Okhli et al., 2020). In another study researchers reported that

using of fennel seed extract at 100-800 ppm in soybean oil reduced totox values in oil samples. The extract at levels of 300 and 400 ppm showed higher antioxidant activity than BHT and BHA (Mazaheri et al., 2014).

3.3. Colour properties of the samples

The colour attributes of the oil are the main criteria affecting consumer acceptance. The colour data of the samples are displayed in Figure 4. As a measure of the colour (*L**, brightness/darkness and *b**, blueness/yellowness) of the oil. All treatments showed darkening over time (ie, decreasing *L**). *L** value was 54.1±0.14 at the beginning of the storage process for all samples. Then *L** decreased to 49.98, 53.70, 50.68, 51.71, 50.80 and 51.64 after 15 days under heating and light conditions for C, T, GC, GCT, GSH and GSHT, respectively. At the same time, the initial *b** value of 3.6 increased to 5.68, 4.13, 5.04, 4.92, 5.10 and 5.19 for C, T, GC, GCT, GSH and GSHT, respectively. The yellow colour of these oils is defined by increasing of *b** over time during storage, because of the natural carotenoids in the sunflower oil.

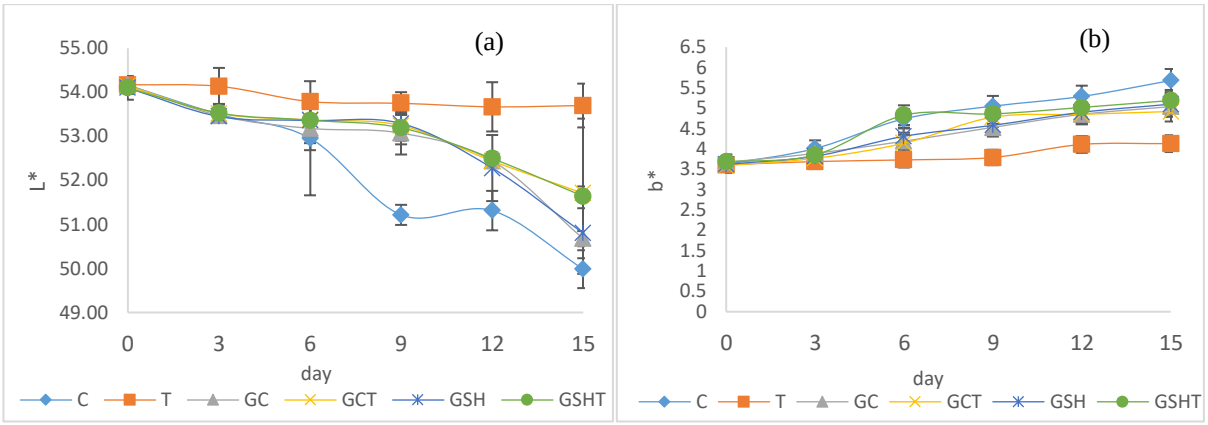

Figure 4. Changes in the *L** (a) and *b** (b) value in sunflower oil during 15 days at 50˚C. C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3

This study states that significant difference between the *b** value of GC and GSH samples. However there was no difference for *L** value ($p>0.05$). In the control sample, while the *L** value decreased dramatically, the *b** value increased. Colour value is an important criterion especially in frying oils (Erol et al., 2022). A colour change was observed in frying oil during oxidation in a study. In another study, it was observed that the *L** value decreased rapidly and the *b** value increased significantly (Urbančič et al., 2014).

Principal component analysis (PCA) was utilized to demonstrate a better explanation of the chemical composition of the sunflower oil with different additives. Figure 5. shows the correlation biplot for the composition of oil samples. The plot indicates that the first two components (F1 and F2) account for 92.09 percent of the required information regarding the differences between oils oxidation profile. Control sample replaced in the first region of the coordinate system, GSH alone fell in the same region as the C, GSHT found its place in the 2nd region with T. GCT and T added oil groups fell into separate regions on the graph, although they had the lowest value when looking at oxidation criteria. This indicates that they have close affects on oxidation, especially in maintaining the *L** value. Additionaly there was positive correlation between *PV* and *FFA* (r=0,94), *b** and *P-Av* (r=0,92), *P-Av* and *FFA* (r=0,93) while *L** and *b** showed negative correlation (r=0,93).

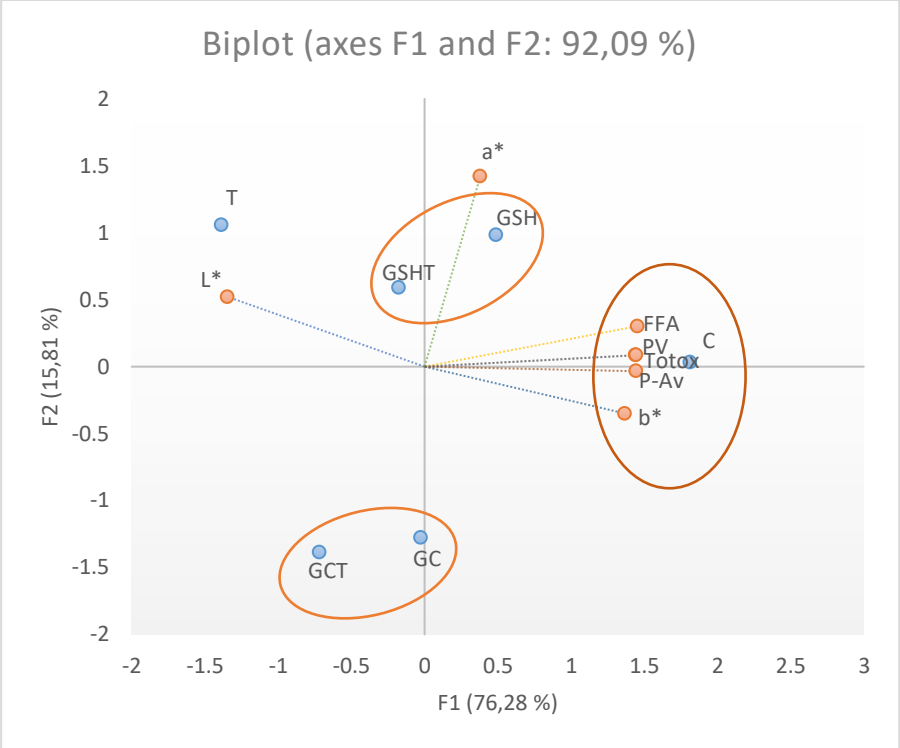

Figure 5. Principle component analysis according to the sunflower oil samples based composition of oils et the end of storage, C, GSH (40 mg/L), GC (40 mg/L) and TBHQ (200 mg/L), GCT (40 mg/L+40 mg/L) and GSHT (40 mg/L+40 mg/L)

Table 1. Fatty acid composition of sunflower oil by GC/MS

	C16:0 (Palmitic acid)				C18:0 (Stearic acid)				C18:1 (Oleic acid)				C18:2 (Linoleic asit)			
	IS		ES		IS		ES		IS		ES		IS		ES	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C	6,72^{a}	±0,97	7,61^{ab}	±0,05	2,84^{a}	±0,45	3,2^{a}	±0,02	31,65^{b}	±0,03	32,45^{a}	±0,08	55,96^{a}	±0,28	55,6^{ab}	±0,37
T	7,48^{a}	±0,06	7,46^{c}	±0,03	3,16^{a}	±0,03	3,17^{a}	±0,05	32,01^{a}	±0,1	31,93^{b}	±0,11	56,39^{a}	±0,25	56,39^{a}	±0,35
GC	7,50^{a}	±0,07	7,51^{bc}	±0,09	3,16^{a}	±0,02	3,20^{a}	±0,02	32,05^{a}	±0,09	32,18^{ab}	±0,27	56,27^{a}	±0,17	55,83^{ab}	±0,43
GCT	7,48^{a}	±0,03	7,49^{bc}	±0,08	3,19^{a}	±0,02	3,19^{a}	±0,04	31,88^{a}	±0,05	31,89^{b}	±0,19	56,15^{a}	±0,01	56,17^{a}	±0,3
GSH	7,46^{a}	±0,05	7,66^{a}	±0,00	3,19^{a}	±0,02	3,25^{a}	±0,00	32,01^{a}	±0,11	32,47^{a}	±0,00	56,24^{a}	±0,27	55,35^{b}	±0,00
GSHT	7,44^{a}	0,02	7,48^{bc}	±0,02	3,17^{a}	±0,02	3,18^{a}	±0,01	31,87^{a}	±0,00	32,08^{a}	±0,09	56,18^{a}	±0,02	56,36^{a}	±0,06

IS: initial storage, ES: end of storage, C (control), T (TBHQ), GC (γ-glutamyl cysteine), GCT (γ-glutamyl cysteine+TBHQ), GSH (glutathione), GSHT (glutathione+TBHQ). Mean ±SD, *n*=3

3.4. Fatty acide profile

Refined sunflower oil contains approximately 15% saturated, 85% unsaturated fatty acids and forms of 14-43% oleic and 44-75% linoleic acids in unsaturated fatty acid content. Fatty acid composition of oil samples shown in Table 1.

Adding 200 mg TBHQ appears benificial in shielding the oil from oxidation, based on comparing the results before and after storage for all fatty acids. The level of oleic acid is the measure of oxidation. When GC was compared to GSH, it was shown that GC inhibited oleic acid growth ($p>0.05$). Palmitic acid and linoleic acid showed no appreciable change before and

after storage. The results show lower levels of stearic, palmitic and linoleic fatty acids compared to test conducted with sunflower oil and higher levels of oleic acid (Akkaya, 2018; Wang et al., 2018).

Loh et al (2006) investigated the effect of synthetic antioxidants (100-750 mg/L) on palm oil during a 5-week storage period. They determined the synthetic antioxidants effect on fatty acids to be in order of vitamin E<BHT<TBHQ<BHA<PG (Propyl gallate). Considering the IC_{50} values in our current study, we predict that increasing the amount of GC and GSH in future studies may decrease the fatty acid composition.

4.Conclusions

In this study, the antioxidant affect of two thiols, γ-glutamyl cysteine and glutathione, in sunflower oil under accelerated storage was studied. The findings of this study demonstrated that GSH and GC worked affectively as antioxidants in sunflower oil when it was stored. The antioxidant of GC, the precursor of GSH, was shown to be more important than GSH. When combined with the synthetic antioxidant, the use of GC and GSH produced a synergistic affect. This indicates that the synthetic antioxidant level in sunflower oil can be reduced by using GSH and especially GC as a curative strategy. It is estimated that these two compounds, which are affective even at low concentrations, will give affective results alone like TBHQ when the amount in the oil is increased.

5.References

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Huseyin Erten, methodology, analysis, data analysis, editing draft, Patrick Fickers editing draft, Serkan Selli methodology, editing draft. Mümine Guruk, investigation, methodology, validation, formal analysis, writing-original draft.

Statements and declarations

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