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Inhibition of the Oxidation of Corn Oil Stripped of Tocopherols and Refined Olive Oil by Thiols

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Abstract: The ability of *N*-acetyl-cysteine and glutathione to inhibit the oxidation of corn oil stripped of tocopherols and refined olive oil was examined.

The oxidative stability of corn oil stripped of tocopherols at 50 °C, 120 °C and 180 °C was evaluated. The absorbances at 234 nm and 270 nm, and *p*-anisidine value were monitored. *N*-acetyl-cysteine and glutathione inhibited the oxidation of oil, *N*-acetyl-cysteine at a higher degree. In comparison to BHA at 200 mg/L, both thiols at 20-40 mg/L exhibited lower (50 °C and 120 °C) or similar (180 °C) antioxidant activities. Compounds similar to the two thiols but not containing –SH group, *N*-acetyl-serine and oxidized glutathione, exhibited very low or no antioxidant activities. The oxidative stability of refined olive oil at 20 °C was determined by monitoring the peroxide value. *N*-acetyl-cysteine and glutathione at 20 mg/L exhibited antioxidant activities comparable to that of BHA at 200 mg/L.

Present results show the ability of *N*-acetyl-cysteine and glutathione to inhibit the oxidation of corn oil stripped of tocopherols and refined olive oil indicating that thiols can inhibit the oxidation of any oil.

Keywords: *N*-acetyl-cysteine, glutathione, thiols, oxidation, oil.

INTRODUCTION

SH-containing amino acids and peptides are natural components in our diet and play significant physiological roles *in vivo* as nucleophiles and scavengers of free radicals [1]. Many foods contain glutathione at various levels, since it is present in most plant and animal tissues [2, 3]. *N*-acetyl-cysteine is an excellent nutritional source of cysteine for humans [1]. It has been proposed for use in foods for particular nutritional uses and for special medical purposes [4].

Lipid oxidation is a major cause of spoilage in foods and its products are potentially toxic. Antioxidants are widely used in many foods to prevent fat rancidity. Synthetic antioxidants such as butylated hydroxyanisole (BHA) are widely used because they are effective and cheaper than natural ones. However, the safety and toxicity of synthetic antioxidants have raised important concerns. Hence, considerable interest has been expressed for the use of natural antioxidants because of their potential nutritional and therapeutic properties [5]. The antioxidant activities of SH-containing amino acids and peptides against the peroxidation of lipids have been reported [6-9].

The main effort of present study was to evaluate the ability of *N*-acetyl-cysteine, and glutathione to inhibit the oxidation of corn oil stripped of tocopherols and refined olive oil.

MATERIALS AND METHODS

Reagents and Oils

N-acetyl-cysteine, *N*-acetyl-serine, glutathione, oxidized glutathione and butylated hydroxyanisole (BHA) were purchased from Sigma (St. Louis, MO, USA). Corn oil stripped of tocopherols was purchased from INC Biochemicals, Inc. (Aurora, OH, USA). Olive oil used was a refined one (Elais, Athens, Greece).

Oxidation of Corn Oil Stripped of Tocopherols

The ability of amino acids/peptides to inhibit the oxidation of corn oil stripped of tocopherols was evaluated at 50, 120 and 180 °C. Each amino acid/peptide or BHA was added in solid form and dissolved using ultrasonic bath for 10 min. A standard amount of corn oil stripped of tocopherols was put into glass bottles of 5 mL capacity (D=2.3 cm, h=2.4 cm). Two milliliters were used for experiments at 50 °C, while 1 mL for those at 120 or 180 °C. Bottles were put open in an oven at the appropriate temperature. Bottles were taken at periodic intervals and the oxidative stability of oil was evaluated by measuring the absorbances at 234 nm and 270 nm, and by determining the *p*-anisidine value.

For the UV absorbances, 10 μL of each sample were mixed with 10 mL of isooctane and the absorbances were followed at 234 nm and 270 nm using cells of 1 cm. Isooctane was used as a blank. When it was necessary, the mixtures were diluted more with isooctane in order to take a measurable absorbance, which was then corrected according to this extra dilution [10]. For *p*-anisidine value, 0.1-0.2 g of each sample to the nearest 0.001 g were put into a 5 mL volume flask and diluted to volume with iso-octane. The absorbance at 350 nm (Ab) was measured using cells of 1 cm and the solvent as a blank. Five milliliters of each oil solution or solvent were mixed with 1 mL *p*-anisidine reagent (2.5 g/L in acetic acid). After exactly 10 minutes the absorbance at 350 nm (As) of each sample mixture was measured using the solvent mixture as blank. The *p*-anisidine value (*p*-AV) was calculated by the formula *p*-AV=5x(1.2 As-Ab):m, where As

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is the absorbance of oil solution after reaction with *p*-anisidine reagent, Ab is the absorbance of the solution of the oil, m is the mass, in gr, of the test portion [10]. In all cases, the absorbance was measured using a Jenway 6505 UV/Vis spectrophotometer (Jenway, Dunmow, England).

Oxidation of Refined Olive Oil

The ability of *N*-acetyl-cysteine and glutathione to inhibit the oxidation of refined olive oil was evaluated at 20 °C. Each thiol or BHA was added in solid form and dissolved using ultrasonic bath for 10 min. Thirty mL of refined olive oil were put in glass bottles of 30 mL capacity (D=2.5 cm, h=6 cm). Bottles were stored open at 20 °C. Bottles were taken at periodic intervals and the oxidative stability of olive oil was evaluated by determining the peroxide value (PV).

For peroxide value determination, a known weight of sample (about 2 g) was weighed into a flask with cup, and air oxygen was removed using nitrogen gas. The sample was dissolved in 10 mL chloroform; 15 mL acetic acid and 1 mL saturated solution of potassium iodine (KI) were added, and mixed for 1 min. The mixture was left in the dark at room temperature for 5 min. Then, distilled water (75 mL) was added and the mixture shaken vigorously. Freshly prepared starch solution (1 %, 1 mL) was added and the resulting solution titrated with 0.002 N solution sodium thiosulphate ($Na_2S_2O_3$) until the colour became clear. A blank containing no sample was also prepared. The peroxide value was calculated using the formula PV=[1000x(Vs-Vb)xN]/W, where Vs and Vb is the volume of $Na_2S_2O_3$ used for titration of sample and blank respectively, N is the normality of $Na_2S_2O_3$ and W is the amount of sample (g). PV is expressed as milliequivalents (meq) of active oxygen per kg of sample [7, 10].

Statistical Analysis

Each experiment was repeated three times, and results reported here are the means of the three runs. The one way analysis of variance (ANOVA), using the Duncan test at a level of significance $p<0.05$ was used for the statistical analysis (SPSS 11.5).

RESULTS AND DISCUSSION

The antioxidant activities of *N*-acetyl-cysteine, *N*-acetyl-serine, glutathione and oxidized glutathione were evaluated in tocopherol free corn oil at 50, 120 and 180 °C. Moreover, BHA was used for comparison. Both primary and secondary oxidation products were followed. Conjugated dienes (A234 nm), conjugated trienes (A270 nm), and high-molecular-weight carbonyl compounds (*p*-anisidine value) were measured.

The antioxidant activity of N-acetyl-cysteine and glutathione at 50 °C is presented in Table **1**. The absorbance at 234 nm and *p*-anisidine value are presented, while the absorbance at 270 nm was not changed. Both thiols inhibited the increase of the absorbance at 234 nm and *p*-anisidine value during oil storage for up to 14 days. *N*-acetyl-cysteine ap-

Table 1. Inhibition of the Oxidation of Corn Oil Stripped of Tocopherols - A234 nm and *p*-Anisidine Value - by *N*-Acetyl-Cysteine and Glutathione During Storage at 50 °C

Sample	0 days	2 days	4 days	7 days	10 days	12 days	14 days
A234 nm							
Control	0.298^a ± 0.020	0.376^a ± 0.021	0.501^a ± 0.019	0.836^a ± 0.028	1.320^a ± 0.038	2.017^a ± 0.023	2.776^a ± 0.032
BHA, 200 mg/L	0.298^a ± 0.019	0.326^b ± 0.029	0.355^c ± 0.036	0.489^d ± 0.004	0.638^d ± 0.045	0.839^d ± 0.012	0.948^d ± 0.015
NAC, 20 mg/L	0.295^a ± 0.011	0.307^b ± 0.015	0.350^c ± 0.016	0.532^c ± 0.040	0.773^c ± 0.056	0.999^c ± 0.030	1.194^c ± 0.041
GSH, 20 mg/L	0.298^a ± 0.013	0.339^b ± 0.041	0.437^b ± 0.033	0.695^b ± 0.021	1.134^b ± 0.044	1.777^b ± 0.008	2.251^b ± 0.049
***p*-anisidine value**							
Control	11.0^a ± 0.0	11.7^a ± 0.3	12.1^a ± 0.1	13.1^a ± 0.6	16.4^a ± 0.9	21.5^a ± 1.6	34.0^a ± 4.2
BHA, 200 mg/L	11.0^a ± 0.0	11.2^b ± 0.2	11.5^b ± 0.1	11.7^b ± 0.2	12.0^c ± 0.2	12.6^d ± 0.2	13.7^d ± 0.8
NAC, 20 mg/L	11.0^a ± 0.0	11.2^b ± 0.2	11.5^b ± 0.2	12.0^b ± 0.5	13.6^b ± 0.6	15.7^c ± 1.0	19.7^c ± 2.4
GSH, 20 mg/L	11.0^a ± 0.0	11.3^b ± 0.2	$11.8^{a,b}$ ± 0.1	$12.9^{a,b}$ ± 0.6	$15.5^{a,b}$ ± 1.3	19.6^b ± 1.5	28.3^b ± 1.8

Means are given with their standard deviation.
a, b, c, d: Means of each index in every column without common superscript differ significantly at $p<0.05$.
BHA=Butylated hydroxyanisole; NAC=*N*-acetyl-cysteine; GSH=Glutathione.

peared to be more effective that glutathione. At t=12 or 14 days, samples containing 20 mg/L *N*-acetyl-cysteine exhibited absorbances at 234 nm less than 50 % of those of the control samples. However, both thiols, each at 20 mg/L, were less effective than BHA at 200 mg/L. The ability of *N*-acetyl-serine and oxidized glutathione, each at 20 mg/L, to inhibit oil oxidation at 50 ^{o}C was also evaluated at 0, 7 and 14 days. However, both of them were inactive, since they did not affect A234 nm and *p*-anisidine values of samples (data not shown).

The antioxidant activity of *N*-acetyl-cysteine and glutathione at 120 ^{o}C is presented in Table **2**. The absorbances at 234 nm and 270 nm, and *p*-anisidine value are presented. Both thiols inhibited the increase of all three oxidation indexes during oil storage for up to 12 hours. *N*-acetyl-cysteine appeared to be more effective than glutathione. However, both thiols, each at 30 mg/L, were less effective than BHA at 200 mg/L. The ability of *N*-acetyl-serine and oxidized glutathione, each at 30 mg/L, to inhibit oil oxidation at 120 ^{o}C was also evaluated at 0, 3 and 6 hours. Both of them exhibited some antioxidant activity, since all three oxidation indexes were statistically lower than those of control. However, the decrease of three indexes in comparison to those of control was usually less than 10 % with a maximum of 20 %. Their antioxidant abilities were much lower than those of N-acetyl-cysteine and glutathione (data not shown).

The antioxidant activity of *N*-acetyl-cysteine and glutathione at 180 ^{o}C is presented in Table **3**. The absorbances at 234 nm and 270 nm are presented. Both thiols inhibited the increase of these two oxidation indexes during oil storage for up to 120 minutes. *N*-acetyl-cysteine appeared to be more effective than glutathione, while their action was dose

Table 2. Inhibition of The Oxidation of Corn Oil Stripped of Tocopherols - A234 nm, A270 nm and *p*-Anisidine Value - by *N*-Acetyl-Cysteine and Glutathione During Storage at 120 ^{o}C

Sample	0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h	10.5 h	12 h
A234 nm									
Control	0.224^{a} ± 0.021	1.041^{a} ± 0.080	1.515^{a} ± 0.063	1.925^{a} ± 0.107	2.263^{a} ± 0.064	2.507^{a} ± 0.080	2.950^{a} ± 0.041	3.375^{a} ± 0.142	3.579^{a} ± 0.182
BHA, 200 mg/L	0.224^{a} ± 0.021	0.638^{d} ± 0.044	0.873^{d} ± 0.084	1.247^{d} ± 0.104	1.571^{d} ± 0.020	1.727^{d} ± 0.018	1.876^{c} ± 0.051	2.086^{c} ± 0.053	2.371^{d} ± 0.118
NAC, 30 mg/L	0.223^{a} ± 0.020	0.711^{c} ± 0.054	1.173^{c} ± 0.090	1.394^{c} ± 0.121	1.655^{c} ± 0.070	1.815^{c} ± 0.027	1.925^{c} ± 0.041	2.111^{c} ± 0.083	2.588^{c} ± 0.136
GSH, 30 mg/L	0.222^{a} ± 0.022	0.855^{b} ± 0.064	1.331^{b} ± 0.080	1.575^{b} ± 0.149	1.828^{b} ± 0.042	2.019^{b} ± 0.021	2.247^{b} ± 0.079	2.609^{b} ± 0.106	3.095^{b} ± 0.195
A270 nm									
Control	0.091^{a} ± 0.001	0.180^{a} ± 0.005	0.223^{a} ± 0.004	0.307^{a} ± 0.004	0.364^{a} ± 0.016	0.380^{a} ± 0.015	0.441^{a} ± 0.017	0.542^{a} ± 0.012	0.682^{a} ± 0.031
BHA ,200 mg/L	0.091^{a} ± 0.001	0.144^{c} ± 0.014	0.184^{c} ± 0.009	0.204^{c} ± 0.008	0.250^{c} ± 0.004	0.298^{c} ± 0.005	0.321^{d} ± 0.007	0.353^{d} ± 0.012	0.418^{c} ± 0.007
NAC, 30 mg/L	0.091^{a} ± 0.001	0.154^{c} ± 0.006	0.190^{c} ± 0.004	0.221^{c} ± 0.004	0.270^{c} ± 0.018	0.307^{c} ± 0.006	0.341^{c} ± 0.005	0.383^{c} ± 0.008	0.436^{c} ± 0.012
GSH, 30 mg/L	0.092^{a} ± 0.001	0.164^{b} ± 0.009	0.210^{b} ± 0.004	0.249^{b} ± 0.009	0.302^{b} ± 0.003	0.321^{b} ± 0.004	0.379^{b} ± 0.001	0.416^{b} ± 0.006	0.595^{b} ± 0.010
***p*-anisidine value**									
Control	9.8^{a} ± 0.0	50.8^{a} ± 4.4	86.7^{a} ± 2.8	146.0^{a} ± 8.0	184.9^{a} ± 4.7	222.9^{a} ± 4.1	247.0^{a} ± 9.2	323.3^{a} ± 12.4	349.1^{a} ± 6.7
BHA, 200 mg/L	9.8^{a} ± 0.0	21.8^{d} ± 2.1	36.5^{d} ± 0.9	84.7^{d} ± 4.3	134.3^{c} ± 1.5	161.0^{d} ± 3.4	190.1^{c} ± 4.1	236.4^{d} ± 4.8	250.0^{d} ± 8.0
NAC, 30 mg/L	9.8^{a} ± 0.0	32.6^{c} ± 4.1	60.7^{c} ± 1.8	107.8^{c} ± 6.0	163.0^{b} ± 6.2	193.5^{c} ± 10.0	202.2^{b} ± 6.9	256.2^{c} ± 7.9	272.0^{c} ± 6.0
GSH, 30 mg/L	9.8^{a} ± 0.0	42.2^{b} ± 5.3	71.7^{b} ± 4.2	120.3^{b} ± 5.3	169.3^{b} ± 4.7	204.5^{b} ± 10.9	214.2^{b} ± 14.0	302.4^{b} ± 7.5	314.2^{b} ± 8.4

Means are given with their standard deviation.
a, b, c, d: Means of each index in every column without common superscript differ significantly at $p<0.05$.
BHA=Butylated hydroxyanisole; NAC=*N*-acetyl-cysteine; GSH=Glutathione.

Table 3. Inhibition of the Oxidation of Corn Oil Stripped of Tocopherols - A234 nm and A270 nm - by *N*-Acetyl-Cysteine During Storage at 180 ^oC

Sample	0 min	30 min	60 min	120 min
234 nm				
Control	0.418^a ± 0.003	1.520^a ± 0.037	1.772^a ± 0.020	2.490^a ± 0.008
BHA, 200 mg/L	0.414^a ± 0.006	0.801^c ± 0.020	1.057^c ± 0.004	2.005^b ± 0.049
NAC, 20 mg/L	0.415^a ± 0.004	0.930^b ± 0.011	1.144^b ± 0.019	1.897^c ± 0.019
NAC, 40 mg/L	0.418^a ± 0.002	0.827^c ± 0.040	1.002^d ± 0.060	1.443^d ± 0.009
270 nm				
Control	0.129^a ± 0.002	0.365^a ± 0.008	0.461^a ± 0.017	0.534^a ± 0.007
BHA, 200 mg/L	0.129^a ± 0.001	0.238^b ± 0.019	0.289^b ± 0.002	0.376^b ± 0.014
NAC, 20 mg/L	0.127^a ± 0.001	$0.223^{b,c}$ ± 0.004	0.299^b ± 0.014	0.366^b ± 0.020
NAC, 40 mg/L	0.127^a ± 0.000	0.216^c ± 0.003	0.261^c ± 0.021	0.342^c ± 0.023
234 nm				
Control	0.418^{a*} ± 0.003	1.520^a ± 0.037	1.772^a ± 0.020	2.490^a ± 0.008
BHA, 200 mg/L	0.414^a ± 0.006	0.801^d ± 0.020	1.057^d ± 0.004	2.005^b ± 0.049
GSH, 20 mg/L	0.417^a ± 0.003	1.030^b ± 0.050	1.465^b ± 0.033	2.041^b ± 0.022
GSH, 40 mg/L	0.416^a ± 0.006	0.948^c ± 0.051	1.323^c ± 0.047	1.838^c ± 0.032
270 nm				
Control	0.129^a ± 0.002	0.365^a ± 0.008	0.461^a ± 0.017	0.534^a ± 0.007
BHA, 200 mg/L	0.129^a ± 0.001	$0.238^{c,d}$ ± 0.019	0.289^d ± 0.002	0.376^c ± 0.014
GSH, 20 mg/L	0.130^a ± 0.001	$0.284^{b,c}$ ± 0.009	0.347^b ± 0.016	0.424^b ± 0.002
GSH, 40 mg/L	0.130^a ± 0.002	0.251^c ± 0.033	0.317^c ± 0.011	0.365^c ± 0.008

Means are given with their standard deviation.
a, b, c, d: Means of each index in every tetrad and column without common superscript differ significantly at $p<0.05$.
BHA=Butylated hydroxyanisole; NAC=*N*-acetyl-cysteine; GSH=Glutathione.

Table 4. Inhibition of the Oxidation of Refined Olive Oil - Peroxide Value- by *N*-Acetyl-Cysteine and Glutathione During Storage at 20 °C

Sample	0 days	56 days	112 days	168 days	224 days	252 days	280 days	308 days
Control	1.9^{a} ± 0.2	2.6^{a} ± 0.2	3.0^{a} ± 0.2	3.3^{a} ± 0.1	3.7^{a} ± 0.2	3.9^{a} ± 0.2	4.1^{a} ± 0.2	4.4^{a} ± 0.2
BHA, 200 mg/L	1.9^{a} ± 0.2	2.3^{a} ± 0.2	$2.7^{a,b}$ ± 0.1	$3.1^{a,b}$ ± 0.2	$3.4^{a,b}$ ± 0.2	3.5^{b} ± 0.1	$3.6^{b,c}$ ± 0.2	3.8^{b} ± 0.2
NAC, 20 mg/L	1.9^{a} ± 0.2	2.1^{a} ± 0.3	2.4^{b} ± 0.2	2.8^{b} ± 0.2	3.1^{b} ± 0.2	3.2^{c} ± 0.0	3.4^{c} ± 0.2	3.5^{b} ± 0.2
GSH, 20 mg/L	1.9^{a} ± 0.2	2.2^{a} ± 0.2	$2.6^{a,b}$ ± 0.2	$3.0^{a,b}$ ± 0.2	$3.4^{a,b}$ ± 0.2	3.5^{b} ± 0.2	3.6^{b} ± 0.2	3.7^{b} ± 0.2

Means are given with their standard deviation.
a, b, c: Means in every column without common superscript differ significantly at $p<0.05$.
BHA=Butylated hydroxyanisole; NAC=*N*-acetyl-cysteine; GSH=Glutathione.

dependent. *N*-acetyl-cysteine at 20 mg/L and glutathione at 40 mg/L exhibited antioxidant activities comparable to that of BHA at 200 mg/L, while N-acetyl-cysteine at 40 mg/L higher than the synthetic antioxidant. The ability of *N*-acetyl-serine and oxidized glutathione, each at 40 mg/L, to inhibit oil oxidation at 180 °C was also evaluated at 0, 30 and 60 minutes. However, both of them were inactive, since they did not affect A234 nm and A270 nm of samples (data not shown).

N-acetyl-cysteine and glutathione was also examined as inhibitors of the oxidation of refined olive oil at 20 °C. Primary oxidation products were followed by determining conjugated dienes (peroxide values). The antioxidant activity of *N*-acetyl-cysteine and glutathione is presented in Table **4**. Both thiols, at 20 mg/L, inhibited the increase of peroxide values during olive oil storage (period 252-308 days). They exhibited antioxidant activities comparable to that of BHA at 200 mg/L. *N*-acetyl-cysteine appeared to be more effective than glutathione.

Present results show that *N*-acetyl-cysteine and glutathione are inhibitors of the oxidation of corn oil stripped of tocopherols and refined olive oil and that they are effective at 50 °C, 120 °C and 180 °C, indicating the spectrum of their abilities. In oils used, *N*-acetyl-cysteine and glutathione exhibited antioxidant activities lower or similar to those of BHA. However, BHA was used typically at 200 mg/L, while thiols at much lower concentrations since it has been taken into account their natural presence in foods. Glutathione is present in many foods at various levels, while some fruits and vegetables contain small amounts of *N*-acetyl-cysteine [2, 3]. Results also show that compounds similar to *N*-acetyl-cysteine and glutathione but not containing –SH group, *N*-acetyl-serine and oxidized glutathione, exhibited very low or no antioxidant activities.This indicates that the –SH group is essential for antioxidant activities of the two thiols in oils.

Previously, it was reported that *N*-acetyl-cysteine and glutathione inhibit the oxidation of corn oil [8, 10]. Present results show the ability of thiols to inhibit the oxidation of corn oil stripped of tocopherols and refined olive oil indicating that they can inhibit the oxidation of any oil.

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