EFFECTS OF ASCORBYL PALMITATE AND METAL IONS ON OXIDATION OF SUNFLOWER OIL UNDER ACCELERATED OXIDATION CONDITIONS

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ABSTRACT

In this study, oxidative stability of sunflower oil was studied under accelerated oxidation conditions. First, refined commercial sunflower oil was examined for its composition of fatty acids and tocopherols. Then, oil samples were prepared with Fe^{+2} and Cu^{+2} ions (0, 0.15 and 0.3 mg/kg) and ascorbyl palmitate (0, 200 and 400 mg/kg). Oil samples were kept at different temperatures (30, 50 and 70°C) for 20 days. Oxidation parameters includingperoxide value, malonaldehyde concentration and hexanalcontent were periodically followed during the storage to investigate the effects of metal ions and ascorbyl palmitate on oxidation of sunflower oil. Results showed that temperature had significant effects on elevation of all oxidation parameters studied($P<0.05$). It was also found that the concentrations of metal ions and ascorbyl palmitate significantly affected the oxidation of sunflower oil. Addition of 400 mg/kg ascorbyl palmitate restricted increment of peroxide value in both $Fe^{2+}$and Cu^{2+} added samples. While Fe^{2+} significantly increased the hexanal content, the presence ofCu^{2+} increased both hexanal and malonaldehyde values in sunflower oil during oxidation. In samples held at lower temperatures, the hexanal content was almost steady but dramatically increased at higher temperatures. It is concluded that hexanalcontent could be well considered as an indicator of oil oxidation along withmalonaldehyde concentration.

Keywords: Sunflower oil, hexanal, malonaldehyde, peroxide value, oxidative stability.

INTRODUCTION

Oil oxidation has been long recognized for many years butits mechanism is still under investigation. Many researchers investigated the effects of storage conditions on oil oxidation. The primary oxidation products are determined to be hydroperoxides, which are generally unstable, then forming the secondary oxidation products including alkanes, alcohols, aldehydes and acids(Shahidi, 1998; Choe and Min, 2006; Porter, 2013). Many of these chemicals are highly reactiveand may initiate the oxidation reactions on their own. Oxidation reactions and resulting chemicals may contribute to the pathogenesis of cancer, atherosclerosis, heart and allergic diseases (HalliwellandGutteridge, 2015).Therefore, oxidative stability of oils is a concern because of economic and quality loss caused by oxidation (Yanishlieva and Marinova, 2001; Matthäus*et al.,* 2010).

In general, the higher level of unsaturation in oils createsmore susceptibility to oxidative deterioration. Factorssuch as oxygen concentration, presence of antioxidants, metal contaminants, hydroxy compounds and enzymes, exposure to the light and elevated temperaturesalsoinfluence the oxidative stability of oils (Yanishlieva-Maslarova, 2001; Jakeria*et al.,* 2014; Johnson and Decker, 2015).Oxidative stability is the resistance of oils to oxidation during processing and storage (Guillen and Cabo, 2002; Walallawita*et al.,*2016). Resistance to oxidation can be defined as the time period necessary to attain to a critical point of oxidation, which is associated with an evidentsensorial change or anincrease in the oxidation rate (Silva *et al.,* 2001).

Oxidative deterioration can be followed by determination of some quality parameters to estimatethe shelf life of oils (Hamilton, 1994; Diridi*et al*., 2016).Generally, effects of oxidation can be evaluated by acid value (AV), representing hydrolytic reactions; peroxide value (PV), representing conjugated diene and triene acids as indicators of primary oxidation;malonaldehyde (MAD), anisidine and thiobarbituric acid reactive substances (TBARS), as indices of secondary oxidation products.Recently, aldehydes such as hexanal (HEX) and propanal have been used as indicators of oil oxidation. HEX is reported to be a strong indicator of oxidation in animal fat due to high content of omega-6 fatty acids while propanalisconsidered as a better indicator in fish lipids due to high content of omega-3 fatty acids (Shahidi, 1998; Ayala et al., 2014).Hexanal have been evaluated as an indicator for late oxidation level. The effect of microwave heating on hexanal contents of hazelnut, olive, soybean and sunflower oils have been reported by Javidipour*et al.* (2016). They noted that hexanal could be considered as a parameter for evaluation of the quality of oils exposed to microwave heating.

Ascorbyl palmitate (AP)is a methyl ester of ascorbic acid dissolving in lipids. As it is a powerful antioxidant that naturally hydrolyzes into ascorbic and palmitic acids, its use in food products is favored more

than synthetic antioxidants (Yanishlieva-Maslarova, 2001; Upadhyay*et al.,* 2017).Although antioxidative mechanism of AP is not well known, Lee *et al.* (1997) reported AP's ability of reducing photosensitized oxidation of oils by quenching singlet oxygen. Meanwhile, Coppen (1994) noted AP as trace metal remover or sequester, consequently diminishing formation of peroxides.Upadhyay andMishra (2015) reported synergistic effect of oleoresin rosemary and AP with increasing oxidative stability of sunflower oil tested at low and high temperatures.AsAP recently obtained considerable attention from researchers,its antagonistic effect on oil oxidation was investigated at varying concentrations in the present study.

It is often relied on accelerated oxidation tests to quickly assess the oil stability against oxidation (Coppin and Pike, 2001; Tena *et al.,* 2017).Since oxidation is the major cause of oil degradation, most of the accelerated tests are designed to speed up this process by exposing oil samples to elevated temperatures in the presence of excessive amount of air or oxygen (Paul and Mittal, 1997; Tena *et al.,* 2017).In this study, the effects of Fe^{+2}, Cu^{+2}, AP, storage temperature and time on oxidative stability of sunflower oil (SFO) were investigated by using response surface methodology (RSM) to be able to evaluate the effects of 4 factors at a time with reduced number of experimental runs and a through assesment of the effects of different factors on oxidation parameters.

MATERIALS AND METHODS

Sunflower oil used in this study was purchased from a local market in Van, Turkey. Hexanaland 2-methyl-3-heptanone were obtained from Aldrich Chemical Corp. (WI, USA), malonaldehydebis diethyl acetal (97%) was obtained from Acros Organics (NJ, USA) and tocopherol standards were obtained from Riedel-De Haen AG (Seelze-Hannover, Germany).All reagents were of analytical grade.

Preparation of oil samples: SFO was first analyzed for its initial content of Cu^{+2} and Fe^{+2} ions. Oil samples were, then, mixed with 200 and 400 mg/kg of AP.After that, cupric and ferrous sulfateswere dissolved in SFO samples at concentrations of 0.15 and 0.30 mg/kg. The recovery ratio forboth Cu^{2+} and Fe^{2+}were98% fromthe samples.

Conditions for accelerated oxidation: Prepared samples were exposed to heat in aconvectional oven (EN 400 Y, Nuve, Istanbul, Turkey) for accelerated oxidation. For this purpose, 100 mL of oil samples were poured into transparent glass bottles,the bottles were held in oven set at 30, 50, and 70°Cfor up to 20 days (Basturk*et al.,* 2007).Oil samples were analyzed for PV, MAD and HEX before exposing the samples to the heat and later on$10^{th}$and 20^{th}days of storage for determination of the effects of heating on oxidation of sunflower oil.

Fatty acid composition: Fatty acids were first methyl esterified and then profiled using a gas chromatography (GC), Agilent 6890 series GC (Agilent Technologies, Palo Alto, CA). For that, 0.4 g oil sample was dissolved in 4 mL isooctane, and then methyl esterified in 0.2 mL 2 M potassium hydroxide. Analysis of fatty acid methyl esters (FAME) was performed usingthe GCequipped with a flame ionization detector (FID) and a 60 m capillary column (ID=0.25 mm) coated with 0.25 µm of 50%-cyanopropyl-methylpolysiloxane (J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas at a flow rate of 1.5 mL/min and a split ratio of 1:10. Injector and detector temperatures were 250 and 260ºC, respectively. Oven temperature was set at 120 ºC for 5 min,then increased to 240 ºC at a rate of 15 ºC/min, and hold at that final temperature for 20 min(Basturk*et al.,* 2007). Samples were injected into the column inlet using an Agilent 7683 B series automatic injector. FAMEs were identified by comparison of their retention time and equivalent chain length with respect to the standard FAMEs (47885-U, Supelco). FAMEs were quantified according to their percentage area (AOAC 1990).

Tocopherol analysis: In saponification step, 0.5 g of oil sample was placed in a glass tube, mixed with 1.25 mL 60% KOH and pyrogallol (3:10 in ethanol), and held in a waterbath set at 70°C for 30 min. Then, the sample was cooled, mixed with 7 mL of 5% NaCl and 5 mL of hexane, and held in dark on ice for 30 min. After that, upper part of the sample was transferred to the vaporization pot. Hexane was added twice and then removed under nitrogen for extraction. The remaining material was dissolved again in dichloromethane and methanol (1:1, v/v) and the extract was placed in vial (Suraiet *al.,* 1996). 20 µl of extracted sample was injected into high performance liquid chromatography (HPLC) to determine the tocopherol content. Normal phase was used to analyze tocopherols using a ThermoFinnigan HPLC (ThermoFinnigan, San Jose, CA). The chromatographic separation was achieved with a Phenomatographic Luna silica gel column (4.6 mm i.d. x 250 mm, 5 µm particle size, Phenomenex, Torrance, CA) by using a mobile phase of n-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9 v/v/v) at a flow rate of 1.6 mL/min. Fluorescence detectorwas utilized for excitation (Ex) and emission (Em) at wavelengths of 295 and 330 nm, respectively (Panfili*et al.,* 2003). Calibration was done using standard solutions of tocopherols and tocotrienols.

Peroxide value and concentration of metal ions: PV and metal ions were analysed according to the AOAC (1990) methods. The concentrationsof Cu^{+2} and Fe^{+2}were initially determined in sunflower oil to avoid any misconclusion that might arise from theirintialpresence.

The results showed that SFO were free of any of these ions at the beginning. These ions were added to the samples in a sensitivity level of ±0.005 mg/kg.

Malonaldehydecontent: 0.1 g of oil sample was taken into a sample tube. After adding 1 mL of 10mM phosphate buffer (containing 1.15% KCl), the content was vortexed. 1 mL of this mixture was taken into another tube,then 200 µl of 0.1 mM $FeSO_4$ was added. This mixture was kept in a waterbath at 37°Cfor 1 h. Then, 50 µL of 0.01% buthylatedhydroxytoluene (BHT) and 200 µL of 8% sodium dodecyl sulphatewere added. After that, 1.5 mL of 20% acetic acid and 1.5 mL of 0.8% thiobarbituric acid were added. The tubes were kept in a waterbath at 95°C for 1 h and then cooled in water. 750 µL of this mixture was transferred into centrifuge tube and mixed with 750 µL of methanol. Tubes were centrifuged at 5000 rpm for 10 min and about 600-700 µL of supernatant was taken into vials. 5 µL of this supernatant was used for injection into HPLC equipment. Mobile phase was 50 mM phosphate buffer/methanol (65/35) mixture and flux rate was 1.5 mL/min. Using an Agilent 1100 HPLC equipped with ODS 2 reverse phase column and fluorescent detector, peaks were recorded at Ex:532 and Em:553 wavelengths. Qualitative and quantitative analysis of MAD were determined according to the MAD standard (97% malonaldehydebis-diethyl acetate) (Surai and Speake, 1998).A diagram of MAD concentration was obtained against to peak area to determine the linearity of the calibration curve prepared for MAD analysis. Regression equation for the calibration curve was 'Y=23.136X-11.479' and the regression coefficient (R^2) was 0.99. Repeatability of HPLC method developed for MAD determination was checked by recording more than 10 chromatograms of 3 mg/kg MAD solutions daily prepared at the most convenient experimental conditions. Peak area was recorded on these chromatograms and values determined were regarded as accurate. According to the results obtained, relative standard deviation was 1.41%, which concluded that the repeatability of the method was sufficient.

Hexanalanalysis: Solid Phase Microextraction (SPME) fiber (50/30/20,divinilbenzen/carboxen/polydimethylsiloxane, 2 cm, Supelco Co., Bellefonte, PA, USA) was used in extraction of volatile compounds for determination of HEX. SPME fiber was first conditioned in injection block of GC equipment for an hour at 270°C. For extraction process, 2 g of sample was transferred into 20 mL vial. The vial was closed tightly, mixed and heated by keeping on a magnetic stirrer at 45°C for 5 min. After that, SPME fiber was left into the headspace of the vial and allowed to absorb the volatiles for 30 min at 45°C. After extraction, SPME fiber was immediately injected into Agilent 6890 model GC equipped with FID. For quantitative analysis of HEXin the presence of 2-methyl-3-heptanone, the previously obtained results by GC-SPME showed that linear relationship between these two compounds could be given by a regression equation 'Y=0.3937X' (R^2=0.99).By this method, the threshold level for determination of HEX was reported to be 1.6 ppb (Javidipourand Qian, 2008).

Statistical design and analysis: In this study, 4-factor 3-level central composite design was utilized by MINITAB statistics software. Independent variables were storage temperature (Temp, °C), storage time (Time, day), metal concentration (Met, mg/kg, Fe^{2+}or Cu^{2+}), and AP concentration (AP, mg/kg) (Table 1). Dependent variables were PV, MAD and HEX content. Measurements were done in duplicateseparately for Fe^{2+}or Cu^{2+} added samples. A total of 30 experiments wererun with 6 central points for each metal ion. Analysis of variance (ANOVA) were used at a significance level of 0.05. For those of experimental combinations that were not run, dependent variables were estimated by regression equations obtained based on the experimental data. Fit of the models were evaluated by regression coefficients and lack of fit values. Regression equations were obtained by using following second degree formula based on the significance level of the linear, quadratic and interaction effects of the dependent variables.

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^{\,2} + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

In this equation, Y represents dependent variable, β_o constant value, $\beta_i$coefficients of linear terms, β_{ii} coefficients of quadratic terms and β_{ij}coefficients of interaction terms while X_i and X_j were independent variables.

RESULTS AND DISCUSSION

Composition of fatty acids and tocopherols: Fatty acid composition oforiginal SFOsample wasdetermined. According to the results, there are four major fatty acids in SFO accounting up to 98.4% of the total. These fatty acids were linoleic (C18:2), oleic (C18:1), palmitic (C16:0) and stearic acids (C18:0) in the descending order at a ratio of 62.6, 24.8, 6.3 and 4.7%, respectively. Tocopherol content and composition of SFO was also examined. The results revealed that the total tocopherol concentration of SFO was 531.9mg/kg.Distribution of the tocopherols was α, γ and β-tocopherols in the descending order at concentrations of 510, 13.6 and 8.3 mg/kg, respectively.PV, MAD, and HEX values of all samples were given in Table 2 and Table 3 for Fe^{2+}or Cu^{2+} added samples, respectively. Based on the results, regression equation for each dependent variable was obtained for

each metal ion, seperately. Lack of fit level was not significant for all equations, indicating regression models were sufficient in estimation of dependent variables (Table 4).

Peroxide value: Temperature, storage time and metal ion concentration significantly increased PV compared to that of the control while concentration of AP decreased PV as expected. Statistical evaluation of the data revealed that the most important factors affecting PV were storage time and temperature ($P<0.05$). The linear effect of AP concentration was reductive on PV but not statistically significant. The interactive effect of temperature and storage time was also significant($P<0.05$).In SFO samples containing Fe^{2+}, the interactive effects of Temp*Time, and Time*AP concentration were significant (Table 4).PVof samples stored at 70°C was the highest on 20th day of storage, as expected. Samples including 400 mg/kg AP restricted increment ofPV in both $Fe^{2+}$and Cu^{2+}added samples. Samples containing 400 mg/kg AP showed lower PVcompared to that of samples containing 200 mg/kg AP. Therefore, AP was believed to beeffective in preventing PV increment, although statictically not significant. Higher temperature, longer storage time and highermetalconcentration significantly increased PV while AP limited this increase.

According to Javidipour*et al.* (2015) oils with 400 mg/kg AP had higher tocopherol content, and lower PV and MAD levels compared to the control sample with no AP added during chemical interesterfication and storage at 60°C. The highest PV of Fe^{2+} containing SFO samples was 181.2 meq O_2/kg oil. Comparing the metal ions used, Cu^{2+} ion was seen to be reducing the oxidative stability and increasing PV more evidently compared to Fe^{2+} ion. However, Mancuso *et al.* (1999) found that iron's increased ability to decompose lipid peroxides prevented accumulation and thus led to lower peroxide value. As given in Figure 1, storage temperature and time steadily increased PV as expected, while almost no difference was seen atvarying AP concentrations at any storage temperature.

Malonaldehydecontent: In Fe^{+2} added SFO samples, the lineareffects of storage temperature and APconcentration were statistically significanton MAD content ($P<0.05$). The quadratic effects of temperature and time were also significant on MAD of Fe^{+2} added SFO. In addition, the interactive effect of Temp*Time and Time*AP concentration was significant on MAD of Fe^{+2} added SFO (Table 4). On the other hand, the interactive effects were mostly not significant on MAD of SFO samples. As seen from Figure 2, MAD seems to be increasing initially with increasing temperature but then being denatured at higher temperatures. In addition, MAD content unsteadily but significantly increased during storage especially at high AP concentrations inboth Fe^{2+} and Cu^{2+} added samples.The presence of AP effectively increased MAD concentration indicating pro-oxidative effects at levels studied during late stage of oxidation.Storage temperature was also significant in increase of MAD level, as expected.

In Cu^{2+} containing SFO samples, the linear effects of metal and AP concentration were found significant on MAD content (Table 4). The quadratic effects of all factors were also significant beside with the interactive effects of Time*Met and Time*AP in Cu^{2+} containing SFO samples ($P<0.05$). The concentration of MAD was almost steady during the first 10 days of storage and after that, showed a dramatic increase during the second half of storage at all temperatures (Fig 2a). Use of AP did not show anevident preventive effect on MAD formation. Karabulut (2010) reported a strong synergistic effect for AP in the presence of α-tocopherol and pro-oxidative effect for AP in the absence of α-tocopherol for the oxidation of butteroil triacylglycerols. Lower level of AP concentration was even more effective in terms of reducing MAD formation compared to that of higher level of AP in SFO samples. Beddow*et al.* (2001) noted that AP may be more effective against oxidation at certain concentrations.

HexanalContent: The interactive effects of Temp*Time, Temp*AP, Time*Metand finally Time*AP on the HEX content were significant in Fe^{2+} added SFO samples ($P<0.05$). Increasing levels of these parameters caused significant increments in HEX concentration. In Cu^{2+} containing SFO samples, the HEX content was significantly affected by storage temperature, storage time, Cu^{2+} and AP concentrations ($P<0.05$). Some interactive effects of Temp*Time, Temp*AP, Time*Met and lastly Temp*AP were significant on the level of HEX concentration (Table 4). Initially, HEX concentration was steady but then, dramatically increased toward to the end of storage (Fig 3). Sometimes, the first and secondary oxidation products may be simultaneously formed but sometimes, the secondary products may be formed after the primary oxidation products reach to a certain level of concentration (Guillen and Cabo, 2002). In Cu^{2+} containing SFO samples, the interactive effect of Temp*Time significantly affected the concentration of HEX in almost a linear relationship. In general, HEX content was higherin the samples held at higher temperatures for longer periods. The linear effects of AP concentration, storage temperature and storage time were significant in addition to the interactive effects of Temp*Timeand Time*AP on HEX content of SFO samplesboth with Cu^{2+} and Fe^{2+} (Table 4).In samples held at lower temperatures, the HEX content was almost steady but dramatically increased at higher temperatures. The concentration of AP showed increasing effect on HEXformation especially at high concentrations. Metal concentration showed some increasing effect on HEX content especially with longer expose to high

temperatures. Storage temperature and time seem to be the most important factors increasing HEXcontent (Figure 3). On the other hand, AP showed pro-oxidative effect and increased HEX content especially at high temperatures and with longer storage time (Figure 3).

While fatty acid profile is the most important factor determining the oxidative stability of oils (Shahidi, 1998); the effects of natural antioxidants may not be always preventive against oxidation. For example, α-tocopherol is an effective antioxidant at low concentrations while its higher concentrations might show pro-oxidative effects. Similarly, γ-tocopherol is more effective against oxidation at high concentrations compared to α-tocopherol while α-tocopherol is a stronger antioxidant at low concentrations compared to γ-tocopherol (Fuster*et al.,* 1998). With that in mind, tocopherols may be more effective in preventing oxidation when the concentration ofhydroperoxides reach to a certain level (Blekas*et al.,* 1995). Lampi*et al.* (1997) reported that the amount of unsaturated fatty acids and their degree of unsaturation are the most important factors affecting the oxidation beside other factors such as the position of unsaturated fatty acids in the triacylglcerols and the presence of anti- and pro-oxidants. It is also reported that metal ions may not always show pro-oxidative effects in oils (Rossell, 1998).In addition, all these variations may create even more complex situations with different oils due to diverse fatty acid profiles. Therefore, it is obvious that mechanism of oil oxidation may not be explained via a single model (Adhvaryu*et al.,* 2000). In this study, SFO samples treated with different levels of AP and different metal ions under accelerated oxidation conditions presented distinctdevelopments in oxidation for almost each treatment combinations studied.The overall results showed that AP had reducing effect on peroxide formation while increasing HEX and MAD formation. AP may partially hold metals and oxygen initially at early stages of oxidation, consequently preventing peroxide formation to some extend, but then may become ineffective while secondary oxidation products form.

When PV, MAD and HEX values of SFO stored at 30°C were examined, PV gradually and continuously increased during the storage while MAD content decreased. Similarly, the HEX content increased up to 10 days of storage but then decreased. In SFO samples stored at 50°C, PV and HEX continuously increased during the whole period of storage while MAD content decreased up to 10 days of storage and increased later on. In samples stored at 70°C, PV continuously increased in SFO samples with both metal ions, but MAD content continuously decreased in Cu^{2+} containing samples while it increased after 10 days of storage in Fe^{2+} containing samples. TheHEX content of SFO samples initially increased but then decreased in Cu^{2+} containing samples, and continuously increased in Fe^{2+} containing samples. Fomuso*et al.* (2002) did not observe a significant variation in TBARS content during metal-catalyzed oxidation of structured lipid model emulsion. Since aldehydes formed during oxidation did not respond well in TBARS test (Fomuso*et al.,* 2002), it can be concluded that hexanal content may be a more reliable parameter representing secondary oxidation products in where TBARS is not functional.

Table 1.Independent variables and their levels.

Independent variable	Abbreviation	Levels
Storage temperature (°C)	Temp	30, 50, 70
Storage time (day)	Time	0, 10, 20
Metal ions (mg/kg)	Met	0, 0.15, 0.30
Ascorbyl palmitate (mg/kg)	AP	0, 200, 400

Table 2. Experimental and predicted results for oxidation parameters of Fe^{2+} added SFO.

Sample No	Temp (°C)	Time (day)	Fe^{2+} (mg/kg)	AP (mg/kg)	PV		MAD		HEX	
					Predicted	Experimental	Predicted	Experimental	Predicted	Experimental
1	30	0	0	0	11.13	12.32	1.405	1.426	0.046	0.047
2	30	0	0	400	16.26	12.35	1.411	1.431	-0.002	0.048
3	30	0	0.3	400	16.26	12.83	1.411	1.433	-0.002	0.049
4	30	0	0.3	0	11.13	13.01	1.405	1.426	0.046	0.048
5	30	10	0.15	200	17.19	13.76	0.819	0.877	0.158	0.187
6	30	20	0	400	23.26	17.38	1.421	1.345	0.334	0.339
7	30	20	0.3	0	18.13	23.01	0.875	0.940	0.254	0.280
8	30	20	0	0	18.13	19.15	0.875	0.881	0.026	0.118

9	30	20	0.3	400	23.26	20.21	1.421	1.385	0.562	0.547
10	50	0	0.15	200	8.11	12.54	1.534	1.426	0.094	0.049
11	50	10	0.15	200	48.18	49.35	1.237	1.243	0.340	0.338
12	50	10	0.15	200	48.18	45.63	1.237	1.051	0.340	0.309
13	50	10	0.15	200	48.18	39.72	1.237	1.400	0.340	0.367
14	50	10	0.15	200	48.18	52.64	1.237	1.462	0.340	0.397
15	50	10	0.15	200	48.18	48.01	1.237	1.030	0.340	0.276
16	50	10	0.15	200	48.18	43.89	1.237	1.213	0.340	0.327
17	50	10	0	200	48.18	47.96	1.237	1.218	0.283	0.322
18	50	10	0.15	0	48.18	46.25	1.261	1.265	0.247	0.239
19	50	10	0.3	200	48.18	50.81	1.237	1.229	0.397	0.442
20	50	10	0.15	400	48.18	48.45	1.537	1.541	0.433	0.480
21	50	20	0.15	200	88.26	90.25	1.534	1.651	0.586	0.540
22	70	0	0	0	17.15	11.93	1.389	1.429	-0.016	0.049
23	70	0	0	400	12.02	12.37	1.395	1.436	0.048	0.048
24	70	0	0.3	0	17.15	12.05	1.389	1.423	-0.016	0.050
25	70	0	0.3	400	12.02	12.65	1.395	1.425	0.048	0.050
26	70	10	0.15	200	91.23	97.95	1.063	1.068	0.372	0.340
27	70	20	0	0	170.45	168.30	1.379	1.281	0.404	0.399
28	70	20	0	400	165.32	152.50	1.925	1.929	0.824	0.854
29	70	20	0.3	0	170.45	181.21	1.379	1.364	0.632	0.655
30	70	20	0.3	400	165.32	161.41	1.925	1.994	1.052	1.138

Table 3. Experimental and predicted results for oxidation parameters of Cu^{2+} added SFO.

Sample No	Temp (°C)	Time (day)	Cu^{2+} (mg/kg)	AP (mg/kg)	PV		MAD		HEX	
					Predicted	Experimental	Predicted	Experimental	Predicted	Experimental
1	30	0	0	0	14.62	12.43	1.404	1.412	0.084	0.048
2	30	0	0	400	14.62	11.97	1.410	1.423	0.018	0.047
3	30	0	0.3	400	14.62	12.04	1.418	1.426	0.020	0.048
4	30	0	0.3	0	14.62	12.81	1.412	1.425	0.086	0.049
5	30	10	0.15	200	22.20	10.18	1.121	1.141	0.258	0.211
6	30	20	0	400	29.78	27.59	1.470	1.558	0.214	0.193
7	30	20	0.3	0	29.78	28.73	1.352	1.396	0.246	0.169
8	30	20	0	0	29.78	22.12	1.088	1.113	-0.028	0.114
9	30	20	0.3	400	29.78	32.76	1.734	1.823	0.488	0.434
10	50	0	0.15	200	5.00	12.50	1.566	1.426	0.055	0.049
11	50	10	0.15	200	49.77	43.73	1.293	1.319	0.416	0.429
12	50	10	0.15	200	49.77	50.63	1.293	1.137	0.416	0.378
13	50	10	0.15	200	49.77	46.92	1.293	1.491	0.416	0.479
14	50	10	0.15	200	49.77	52.38	1.293	1.399	0.416	0.382
15	50	10	0.15	200	49.77	56.67	1.293	1.238	0.416	0.420
16	50	10	0.15	200	49.77	45.55	1.293	1.258	0.416	0.464
17	50	10	0	200	49.77	41.90	1.054	1.037	0.347	0.310
18	50	10	0.15	0	49.77	44.48	1.384	1.360	0.338	0.323
19	50	10	0.3	200	49.77	51.56	1.190	1.177	0.485	0.468
20	50	10	0.15	400	49.77	42.33	1.578	1.575	0.494	0.507
21	50	20	0.15	200	94.54	99.37	1.566	1.677	0.543	0.523
22	70	0	0	0	17.90	12.37	1.404	1.420	0.022	0.049
23	70	0	0	400	17.90	12.27	1.410	1.426	0.092	0.048
24	70	0	0.3	0	17.90	12.46	1.412	1.429	0.024	0.050
25	70	0	0.3	400	17.90	13.02	1.418	1.431	0.094	0.049
26	70	10	0.15	200	99.87	112.36	1.121	1.073	0.574	0.574
27	70	20	0	0	181.84	189.11	1.088	1.040	0.530	0.447

28	70	20	0	400	181.84	156.90	1.470	1.371	0.908	0.854
29	70	20	0.3	0	181.84	192.65	1.352	1.300	0.804	0.803
30	70	20	0.3	400	181.84	168.56	1.734	1.613	1.182	1.284

Table 4. Regression coefficients and their significance level of Fe^{2+} and Cu^{2+} containing oil samples.

Independent Variables		Coefficients					
		Fe^{2+} containing samples			Cu^{2+} containing samples		
		PV	MAD	HEX	PV	MAD	HEX
β_0	(Constant)	47.4073^{***}	1.25033^{***}	0.33193^{***}	49.7675^{***}	1.30622^{***}	0.41831^{***}
β_1	(Temp)	37.0194^{***}	0.12250^{**}	0.10722^{***}	38.8372^{***}	-0.03411^{ns}	0.15806^{***}
β_2	(Time)	40.0761^{***}	-0.00472^{ns}	0.24677^{***}	44.7733^{***}	0.00406^{ns}	0.24356^{***}
β_3	(Met)	1.8294^{ns}	0.01350^{ns}	0.05805^{***}	2.1072^{ns}	0.06778^{**}	0.06911^{***}
β_4	(AP)	-2.0600^{ns}	0.13800^{***}	0.09211^{***}	-2.7622^{ns}	0.09728^{***}	0.07844^{***}
β_{11}	(Temp*Temp)	6.5656^{*}	-0.27779^{**}	-0.06903^{**}	11.2686^{**}	-0.18091^{**}	-0.01211^{ns}
β_{22}	(Time*Time)	2.1056^{ns}	0.28821^{**}	-0.03803^{ns}	5.9336^{ns}	0.26359^{***}	-0.11861^{**}
β_{33}	(Met*Met)	0.0956^{ns}	-0.02679^{ns}	0.04947^{ns}	-3.2714^{ns}	-0.18091^{**}	-0.01561^{ns}
β_{44}	(AP*AP)	-1.9394^{ns}	0.15271^{ns}	0.02697^{ns}	-6.5964^{ns}	0.17959^{**}	0.01039^{ns}
β_{12}	(Temp*Time)	36.5738^{***}	0.12625^{**}	0.11043^{***}	37.1969^{***}	-0.03663^{ns}	0.15462^{***}
β_{13}	(Temp*Met)	0.8975^{ns}	0.00187^{ns}	0.01131^{ns}	0.2381^{ns}	-0.00300^{ns}	0.03063^{ns}
β_{14}	(Temp*AP)	-1.8650^{ns}	0.02287^{ns}	0.02731^{**}	-4.0069^{ns}	-0.01450^{ns}	0.03400^{**}
β_{23}	(Time*Met)	1.6837^{ns}	0.01637^{ns}	0.05718^{***}	1.6056^{ns}	0.06375^{**}	0.06738^{**}
β_{24}	(Time*AP)	-2.5663^{*}	0.13538^{***}	0.08843^{***}	-2.8769^{ns}	0.09350^{**}	0.07725^{***}
β_{34}	(Met*AP)	-0.3175^{ns}	-0.00250^{ns}	0.00406^{ns}	0.4394^{ns}	-0.00313^{ns}	0.01625^{ns}
Regression		***	***	***	***	***	***
Linear		***	***	***	***	**	***
Square		ns	**	Ns	ns	***	**
Interaction		***	**	***	***	**	***
R-Sq		0.99	0.90	0.98	0.99	0.89	0.98
R-Sq(adj)		0.99	0.79	0.97	0.98	0.78	0.95
Lack-of-Fit		0.707	0,971	0.489	0.181	0.973	0.269

^{ns}: not significant at 95% (P>0.05); *: significant at 95% (P<0.05); **: significant at 99% (P<0.01); ***: significant at 99.9% (P<0.001).

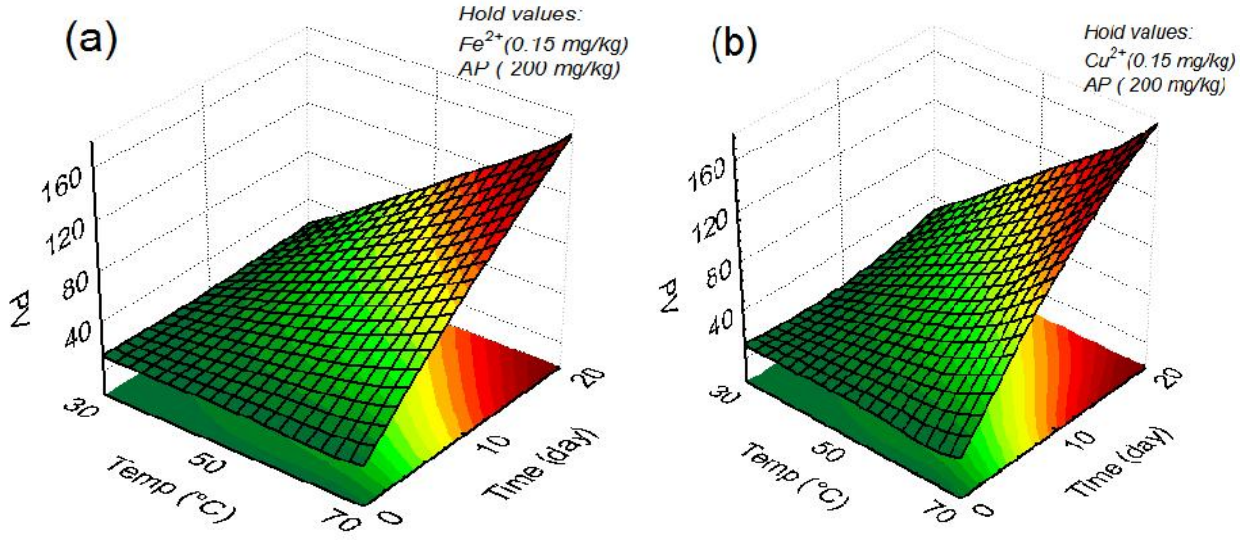


Figure 1. Response surface plots for PV of Fe^{2+} (a) and Cu^{2+} (b) added SFO.

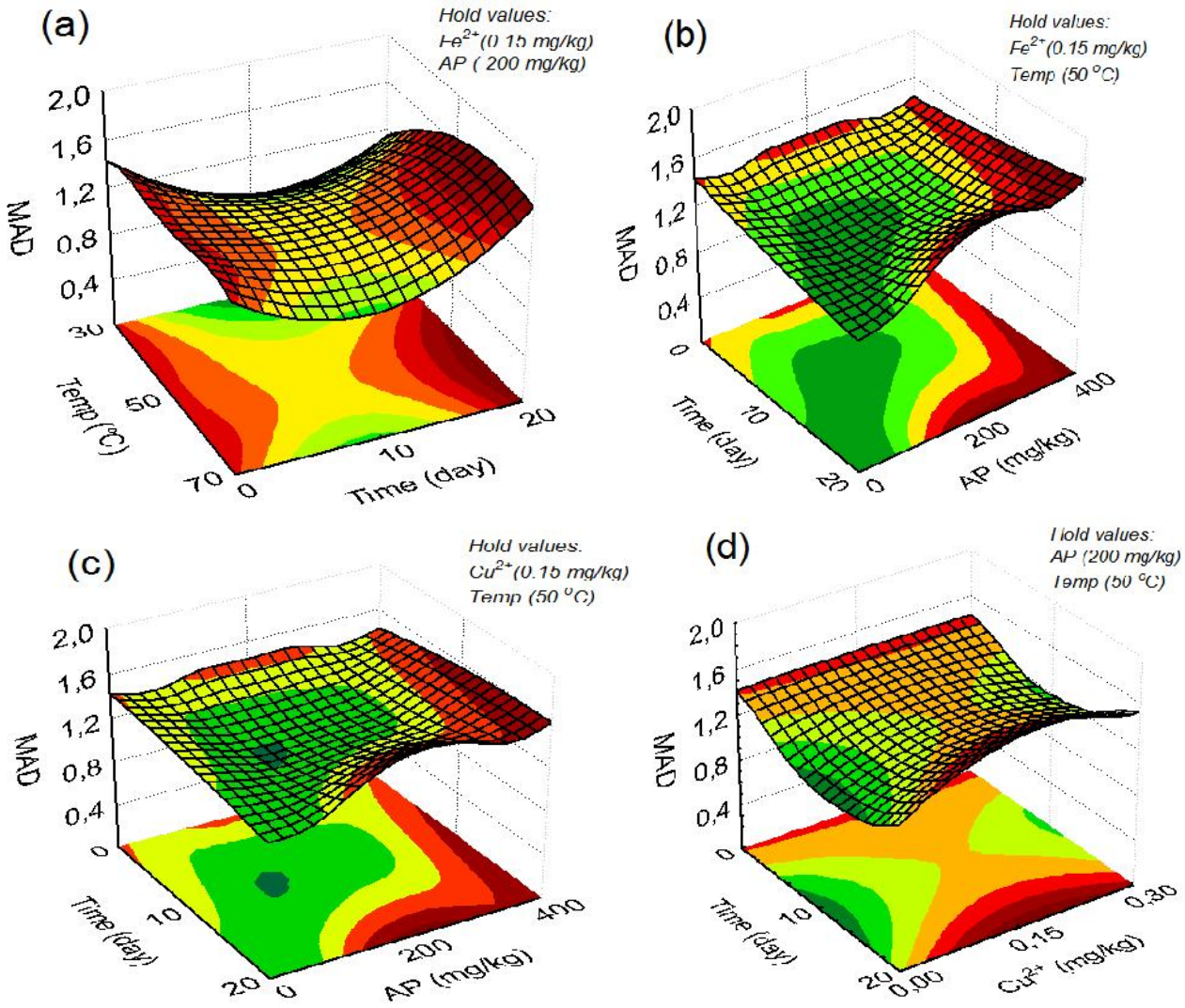
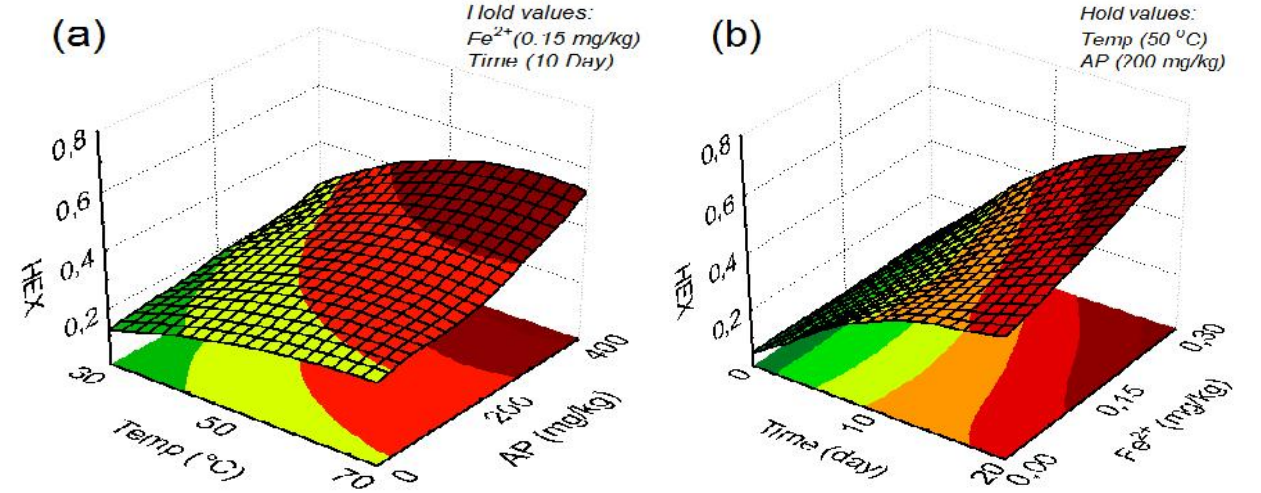


Figure 2. Response surface plots for MAD of Fe^{2+} (a, b) and Cu^{2+} (c, d) added SFO.



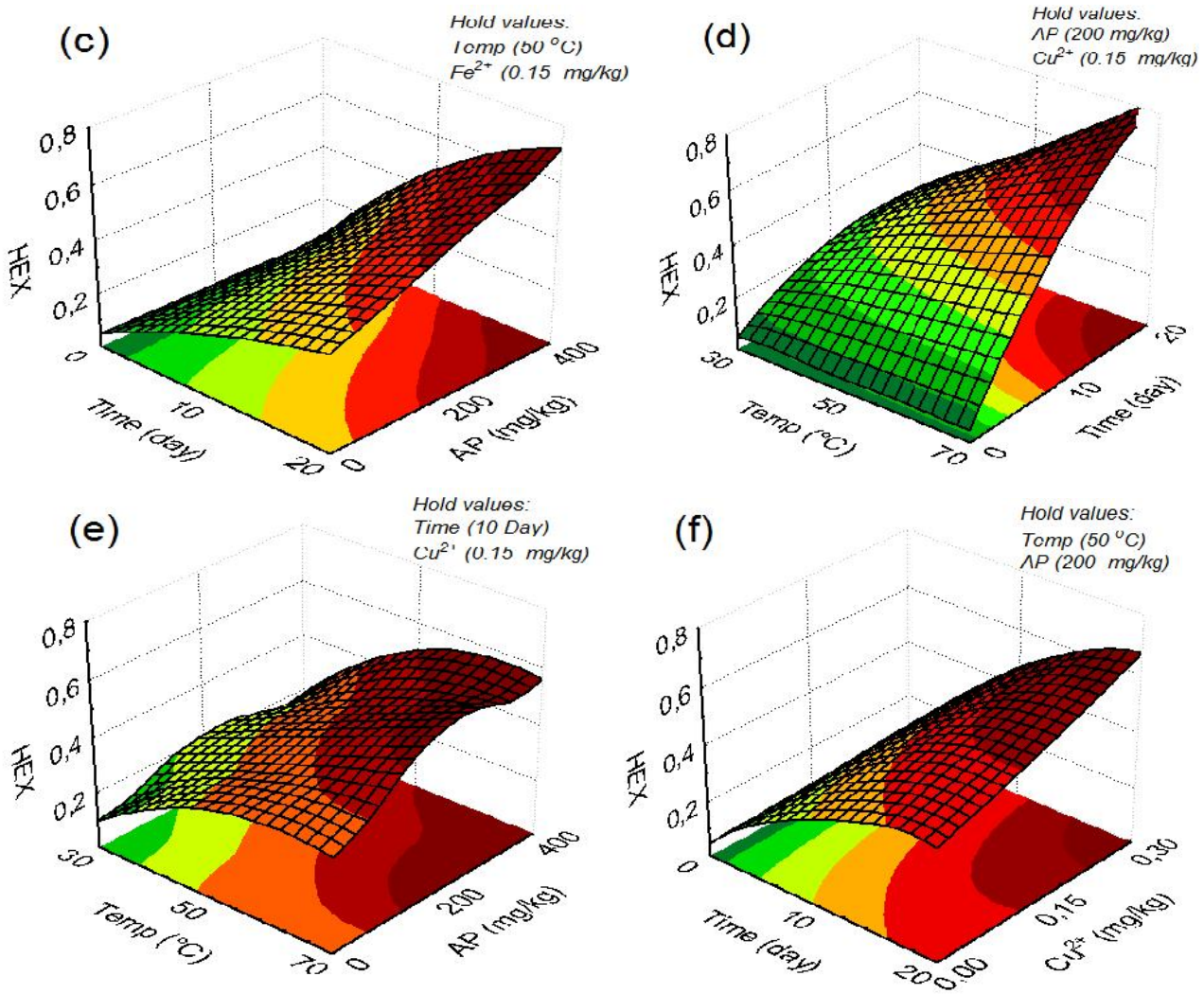


Figure 3.Response surface plots for HEX of Fe^{2+} (a, b, c) and Cu^{2+} (d, e, f) added SFO.

Conclusions: The results obtained showed that the most important factors affecting oil oxidation were storage temperature and time. Among the dependent variables studied, increasing storage temperature and time significantly increased the HEX content in the presence of Fe^{+2} and increased both HEX and MAD values in the presence of Cu^{+2}.Comparing the metal ions tested, Cu^{2+} was seen to be reducing the oxidative stability of SFO more than Fe^{2+}.The addition of AP as an antioxidant was not effectively preventive on oil oxidation as much as expected. The content of natural antioxidants affects oxidation, not always preventive, but in fact, sometimes pro-oxidative.PV values were reduced by addition of AP but this was not the case when MAD and HEX values are considered.

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