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Change in major fatty acid composition of vegetable oil depending on phenolic incorporation and storage period

O.S. Toker[1], I. Ozturk[2], S. Karaman[2*], H. Yalcin[2], A. Kayacier[3], M. Dogan[2] and O. Sagdic[1]

[1]Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Food Engineering Department, 34210 Istanbul, Turkey; [2]Erciyes University, Engineering Faculty, Food Engineering Department, 38039 Kayseri, Turkey; [3]Bursa Technical University, Faculty of Natural Sciences, Architecture and Engineering, Department of Food Engineering, 16190 Bursa, Turkey; skaraman@erciyes.edu.tr

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RESEARCH ARTICLE

Abstract

In the present study, response surface methodology was conducted for the determination of effects of some phenolics (gallic acid, ellagic acid and quercetin) on the major fatty acid composition of vegetable oil prepared by mixing of sunflower and hazelnut oil (50:50, v/v) during storage at a constant temperature (50 °C). In this respect, major fatty acid composition (palmitic, stearic, oleic and linoleic acid) of vegetable oil was determined. Predictive regression equations were constructed for the estimation of each studied parameter (R^2>0.735). Storage period caused a significant increase in palmitic acid, stearic acid and oleic acid content of oil while it caused a decrease in the linoleic acid content of the oil (P<0.01) because of the reactions that occurred in the structure of the oil during storage. In general, gallic acid and quercetin were found to be effective on the preservation of oil against oxidation and addition of gallic acid that retarded the change of major fatty acids composition due to oxidation. Multiple response optimisation was performed by considering the change in all major fatty acids simultaneously. According to the results, addition of ellagic acid, gallic acid and quercetin at the concentrations of 20, 46.7 and 33.3%, respectively, in a phenolic mixture (0.1 g) to the oil sample is convenient for decreasing oxidation.

Keywords: fatty acids, storage, multiple response optimisation, oxidation, phenolics, response surface methodology, vegetable oil

1. Introduction

Edible oils are produced from different plant or plant seeds and they are consumed widely because of their positive health aspects like cholesterol-lowering effects. They are basic sources of mono and polyunsaturated fatty acids having functional properties in human health (Yalcin, 2011). Due to the high double bonds in unsaturated fatty acids present in vegetable based edible oils, oxidation occurs easily and rapidly during the storage. Oxidation can cause lipid peroxidation because of oxygen and a loss in nutritional value in addition to flavour, aroma and texture degradation. Because of the advanced oxidation, the oil becomes inconsumable (Matalgyto and Al Khalifa, 1998). It was reported that the oxidation causes undesired flavour and rancid taste as well as the formation of some potentially toxic compounds that can cause different health problems (Maniak and Targonski, 1996). Because of the structural susceptibility of the vegetable based edible oils, their preservation is quite difficult because of their high unsaturated fatty acid content. The reaction between oxygen and unsaturated fatty acids causes the deterioration of lipids or lipid-containing products (Erkan *et al.*, 2009). It is well known that the addition of antioxidants is quite effective technique to prevent or retard the oxidation of edible oils during storage (Halliwell *et al.*, 1995). There are effective synthetic antioxidants used in the lipids, especially oils, and lipid containing foods like butylated hydroxytoluene, butylated hydroxyanisole (BHA) and tertiary butyl hydroquinone to prevent oxidation (Mohdaly *et al.*, 2010). Goli *et al.* (2005) reported that BHA was removed from the list of GRAS (i.e. generally

recognised as safe; http://tinyurl.com/owf3758). Due to the increased suspects on synthetic antioxidants, researchers have recently started to focus on exploring new natural antioxidant compounds. Many studies reported important results regarding the antioxidant characteristics of phenolic compounds or extracts which are rich in total phenolic content (Armando *et al.*, 1998). As is well known, phenolics are compounds known as secondary metabolites of the plants. Antioxidant activity is their one of the most important biological activities (Cook and Samman, 1996). Many studies have been conducted on the oxidation stability of vegetable oils and many natural extracts have been used for the reterdation of oxidation in the structure of oil (Rehman, 2006; Rehman *et al.*, 2004; Yalcin, 2011; Yalcin *et al.*, 2011). In fact, no study has appeared to focus on the antioxidative effect of phenolic mixtures on the change of major fatty acid composition of vegetable oils. In this study; therefore, it was aimed to investigate the antioxidative effects of phenolic mixtures and storage period on the major fatty acid composition of mixed oil prepared with sunflower and hazelnut oil because the oil mixtures are commonly sold in the marketplace. The sole and mixture effects of the phenolics on fatty acid composition were determined using response surface methodology (RSM). Linear, interaction and quadratic effects of the processing variables were used to predict fatty acid composition of the oil as a function of independent variables using central composite rotatable design. It was also aimed to optimise phenolic substance combination using desirability functions in order to retard change in fatty acid composition of the oils. The results of the present study also provide information about effectiveness of the phenolics or their combinations on fatty acid profile of mixed vegetable oil throughout the storage period.

2. Materials and methods

Materials

Refined sunflower (*Helianthus annuus*) and hazelnut (*Corylus avellana*) oils were procured from local markets and these oils were mixed at the ratio of 50:50 (v/v). Gallic acid, ellagic acid and quercetin were purchased from Sigma-Aldrich (Stockholm, Sweden). Standard fatty acid methyl esters (FAMEs) were provided from ACCU Standard Inc. (New Haven, CT, USA).

Addition of phenolic compounds

The amounts of phenolic compounds added to the 100 ml of mixed oil with solving in ethanol according to the central composite rotatable design are presented in Table 1. All prepared oil samples were stored at 50 °C in a hot-air oven (EN 120; Nüve, Ankara, Turkey) and exposed to constant sunlight and air during the storage period. In addition, mixed oil having no additive was also stored as control sample at the same condition, and analysis of this sample was performed in order to observe the effectiveness of the phenolic compounds added to the oil.

Fatty acid composition

The oil sample was prepared for the analysis after the methylation procedure according to the method described by Yuksel *et al.* (2014). 1 ml of methylated solution was put into gas chromatography vials and 1 µl of the sample was injected immediately. A gas chromatograph (Agilent 6890; Agilent, Chandler, AZ, USA) equipped with a flame ionisation detector and a HP 88 capillary column (100 m × 0.25 mm × film thickness) was used. Injection block temperature was set at 250 °C. The oven temperature was kept at 103 °C for 1 min, then programmed from 103 to 170 °C at 6.5 °C/min, from 170 to 215 °C for 12 min at 2.75 °C/min, finally, 230 °C for 5 min. The carrier gas was helium with a flow rate 2 ml/min and split rate was 1/50. The main fatty acid compositions of the oils (palmitic, stearic, oleic and linoleic acid) were identified according to the retention times of standard FAMEs as percentage (%). The fatty acid analyses of the samples were replicated two times.

Experimental design and statistical analyses

The effect of storage period on the major fatty acid composition of the control sample and significant differences between the samples were determined performing ANOVA by using SPSS 17.0.1 statistical software program (release 17.0.1 for Windows; SPSS Inc., Chicago, IL, USA; Ural and Kilic, 2006). Tukey test was used for determination of the differences among the dependent variables of the oil samples. RSM was used to observe the effect of independent variables (storage period, amount of gallic acid, ellagic acid and quercetin) on the dependent variables (palmitic, stearic, oleic and linoleic acid levels). A rotational central composite design was used and the range of the storage time and phenolic amounts were determined as 0-120 days and 0-0.1 g per 100 ml of oil, respectively. The actual and coded levels of the design variables are shown in Table 1. The actual values were changed to coded variables using the following equation (Wang *et al.*, 2011)

$$C_i = \frac{X_i - X_0}{\Delta X_i} \qquad i = 1, 2, 3...k \qquad (1)$$

where, C_i and X_i are the coded and real value of the independent variables, X_0 represents the real value of the independent variable at the centre point, and ΔX_i is the step change value.

All experiments were replicated three times and there were 5-centre points in the experimental design. The relationship between the independent and dependent variables was

Table 1. Central composite rotatable design for the independent variables (actual and coded levels; oil volume was 100 ml).

Run	Coded level[1]				Actual level			
	X_1	X_2	X_3	X_4	Storage period (day)	Ellagic acid (g)	Gallic acid (g)	Quercetin (g)
1	-1.483	0	0	0	0	0.05	0.05	0.05
2	-1	-1	-1	-1	19.54	0.02	0.02	0.02
3	-1	-1	-1	1	19.54	0.02	0.02	0.08
4	-1	1	1	-1	19.54	0.08	0.08	0.02
5	-1	-1	1	-1	19.54	0.02	0.08	0.02
6	-1	1	-1	1	19.54	0.08	0.02	0.08
7	-1	1	1	1	19.54	0.08	0.08	0.08
8	-1	1	-1	-1	19.54	0.08	0.02	0.02
9	-1	-1	1	1	19.54	0.02	0.08	0.08
10	0	0	0	0	60.00	0.05	0.05	0.05
11	0	0	0	-1.483	60.00	0.05	0.05	0.00
12	0	0	0	0	60.00	0.05	0.05	0.05
13	0	1.483	0	0	60.00	0.10	0.05	0.05
14	0	0	0	0	60.00	0.05	0.05	0.05
15	0	0	0	1.483	60.00	0.05	0.05	0.10
16	0	0	0	0	60.00	0.05	0.05	0.05
17	0	0	-1.483	0	60.00	0.05	0.00	0.05
18	0	0	0	0	60.00	0.05	0.05	0.05
19	0	-1.483	0	0	60.00	0.00	0.05	0.05
20	0	0	1.483	0	60.00	0.05	0.10	0.05
21	1	1	-1	1	100.46	0.08	0.02	0.08
22	1	-1	-1	-1	100.46	0.02	0.02	0.02
23	1	1	1	1	100.46	0.08	0.08	0.08
24	1	-1	1	-1	100.46	0.02	0.08	0.02
25	1	-1	1	1	100.46	0.02	0.08	0.08
26	1	1	1	-1	100.46	0.08	0.08	0.02
27	1	-1	-1	1	100.46	0.02	0.02	0.08
28	1	1	-1	-1	100.46	0.08	0.02	0.02
29	1.483	0	0	0	120.00	0.05	0.05	0.05

[1] X_1 = storage period (day); X_2 = ellagic acid (g); X_3 = gallic acid (g); X_4 = quercetin (g).

explained by the following second degree polynomial equation:

$$Y = \sum_{i=1}^{4}\beta_{ki}X_i + \sum_{i=1}^{4}\beta_{kii}X_i^2 + \sum_{I,j\leq 2}^{4}\beta_{kij}X_iX_j \quad (2)$$

where, y is the dependent variable, β_{ki}, β_{kii} and β_{kij} are the coefficients of linear, quadratic and interaction terms, respectively. X_i and X_j represent the independent variables. The analysis of variance was performed to determine the effect and regression coefficients of linear, quadratic and interaction terms. *P*-values of less than 0.05 were accepted as statistically significant. The model adequacies were examined by R^2 values. The RSM was applied by JMP 5.0.1 statistical package program (Cary, NC, USA).

3. Results and discussion

Fatty acid composition of control mixed oil

Major fatty acid composition of the mixed oil stored at 50 °C for 120 days is shown in Table 2. It's known that the major fatty acids of the sunflower and hazelnut oils are linoleic and oleic acids (Yalcin *et al.*, 2012a,b). For this reason, the major fatty acid composition of the mixed oil was determined as oleic acid with the level of approximately 49.29% and linoleic acids (40.42%) at the beginning of the storage. With the increment of storage period, a significant change was observed in the major fatty acid composition of the oils ($P<0.05$). Palmitic, stearic and oleic acid levels increased with the increment of storage period while the linoleic

Table 2. Effect of storage period on the major fatty acid composition of the control sample.[1]

Storage period (day)	Major fatty acid composition (%)[2]			
	C16:0 (%)[3]	C18:0 (%)	C18:1 n-9 *cis* (%)	C18:2 n-6 *cis* (%)
0	5.47±0.00^{d}	3.28±0.00^{e}	49.29±0.02^{e}	40.42±0.03^{a}
24	5.49±0.01^{d}	3.36±0.00^{d}	49.83±0.03^{d}	39.95±0.06^{b}
60	8.58±0.10^{b}	4.66±0.01^{c}	54.78±0.06^{c}	30.30±0.02^{c}
96	7.98±0.47^{c}	4.92±0.00^{b}	64.70±0.30^{b}	21.73±0.16^{d}
120	10.28±0.03^{a}	5.77±0.01^{a}	67.24±0.29^{a}	15.52±0.03^{e}

[1] Mean ± standard deviation.
[2] C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 n-9 *cis* = oleic acid; C18:2 n-6 *cis* = linoleic acid.
[3] Column values with a different lower-case letter in superscript are significantly different at $P<0.05$.

acid concentration decreased significantly depending on the increasing of storage period ($P<0.05$).

It was reported that the major fatty acids of sunflower oil were palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9) and linoleic acid (C18:2 n-6) with the level of 7.61, 2.79, 18.60 and 70.99%, respectively (Crapiste *et al.*, 1999). Ahn *et al.* (2008) reported that the major fatty acids of sunflower oil were palmitic acid (5.86%), stearic acid (3.28%), oleic acid (37.94%) and linoleic acid (51.40%). In another study, Yalcin *et al.* (2012a) investigated the fatty acid composition of different vegetable oils to determine the effect of fatty acid composition on rheology of oils and reported that the major fatty acids of sunflower oil were 6.36% of palmitic acid, 3.63% of stearic acid, 29.30% of oleic acid and 58.08% of linoleic acid. Crapiste *et al.* (1999) studied the changes in major fatty acid composition of sunflower oil during storage and concluded that oleic acid level increased while the linoleic acid level decreased during storage period. While the oleic/linoleic acid ratio was 0.334 at the beginning of the storage at 67 °C, it ascended until 0.568 at the end of the storage because of a preferential usage of linoleic acid in oxidation reaction (Crapiste *et al.*, 1999).

Major fatty acid composition of mixed oil added with phenolics

Change in major fatty acid composition of the oil samples containing phenolic substance or mixed phenolic substances at different amounts (as presented in Table 1) during storage is shown in Table 3. Palmitic, stearic, oleic, and linoleic acid concentrations of the oil samples changed between 5.33-7.04, 3.31-4.40, 48.28-60.82 and 27.24-40.54%, respectively, which highlighted that a significant difference was observed in the major fatty acid composition of the oil sample ($P<0.05$). In general, palmitic, stearic and oleic acid content increased with storage time while linoleic acid content decreased.

Figure 1 illustrates the change in major fatty acid contents of mixed oil depending on both the storage period and gallic acid content. Only the effect of gallic acid is given since it is the most influential compound among the phenolics analysed in the present study. Palmitic, stearic and oleic acid contents were determined to be 5.33, 3.31 and 49.24% at the beginning of the storage, respectively while the linoleic acid content was 40.31%. After 120 days of storage at 50 °C, palmitic, stearic and oleic acid contents were measured to be 6.74, 3.92 and 58.77%, respectively, while the linoleic acid content was 30.14%. Addition of gallic acid prevented the increase of stearic and oleic acid and decrease of linoleic acid level significantly ($P<0.05$). As stated before, significant correlation among the fatty acid content was observed. In fact, with the increase of oleic acid in the oil, a decrement was observed in the linoleic acid content.

Similar trends in the changes of major fatty acid contents of sunflower oil during storage were reported by Crapiste *et al.* (1999). Neff *et al.* (1994) concluded that the oxidative deterioration of canola triacylglycerols showed a negative correlation with oleic acid and positive correlation with linoleic acid content of oil. They also reported that the oleic and linoleic acid contents of canola oil with very low oxidisability value (0.163) were 81.3 and 6.5% while the canola oil having high oxidisability value (0.412) has 60% oleic acid and 22.4% linoleic acid. Holman and Elmer (1947) investigated the rates of oxidation of unsaturated fatty acids and esters and they reported that the increment in double bonds in a fatty acid increased the oxidation of fatty acid. For that reason, linoleic acid content decreased during the storage because of having more double bonds in its structure compared to oleic acid. In addition to that, Johnson and Kummerow (1957) carried out a research related to chemical changes which take place in edible oil during thermal oxidation and reported that they observed a decrease in iodine value and linoleic acid content. And also, they reported that the percentage of monounsaturated

Table 3. Change in major fatty acid composition of the mixed oil enriched with phenolics for different concentration at different storage periods.[1]

Run	Major fatty acid composition (%)[2]			
	C16:0 (%)	C18:0 (%)	C18:1 n-9 *cis* (%)	C18:2 n-6 *cis* (%)
1	5.33±0.05	3.31±0.01	49.24±0.17	40.31±0.05
2	6.40±0.07	3.41±0.01	48.28±0.01	40.54±0.09
3	5.43±0.05	3.37±0.01	48.94±0.06	40.46±0.01
4	5.47±0.08	3.37±0.05	49.57±0.91	40.31±0.21
5	5.46±0.07	3.35±0.00	49.55±0.24	40.32±0.35
6	5.45±0.07	3.34±0.03	49.81±0.04	40.05±0.08
7	5.45±0.01	3.35±0.00	49.51±0.01	40.37±0.02
8	5.46±0.01	3.31±0.01	49.62±0.19	40.03±018
9	5.57±0.01	3.38±0.00	49.40±0.05	39.95±0.01
10	5.52±0.01	3.39±0.01	49.98±0.06	39.40±0.07
11	5.84±0.22	3.56±0.01	50.57±0.06	38.72±0.21
12	5.83±0.00	3.50±0.01	49.50±0.02	39.64±0.02
13	5.85±0.01	3.50±0.08	50.34±0.02	39.30±0.02
14	5.98±0.03	3.62±0.02	51.30±0.02	37.64±0.02
15	6.05±0.04	3.46±0.01	49.68±0.04	39.37±0.01
16	6.05±0.01	3.74±0.01	52.92±0.07	35.92±0.13
17	6.62±0.66	3.99±0.04	53.75±0.14	33.91±0.55
18	6.55±0.03	3.55±0.00	49.88±0.12	38.55±0.10
19	6.19±0.01	3.63±0.01	51.16±0.03	37.65±0.04
20	5.79±0.02	3.45±0.01	49.90±0.06	39.90±0.10
21	6.66±0.01	4.08±0.01	58.95±0.00	30.09±0.02
22	6.24±0.00	3.83±0.03	56.66±0.05	33.11±0.02
23	5.57±0.04	3.38±0.01	52.54±0.02	38.52±0.18
24	6.68±0.02	3.69±0.02	54.72±0.01	35.03±0.04
25	7.04±0.25	4.40±0.01	60.82±0.10	27.24±0.13
26	6.18±0.06	3.74±0.01	55.76±0.02	34.19±0.03
27	5.78±0.01	3.56±0.00	54.06±0.01	36.60±0.01
28	5.87±0.02	3.49±0.04	53.75±0.01	36.89±0.07
29	6.74±0.20	3.92±0.08	58.77±0.19	30.14±0.50

[1] Mean ± standard deviation.
[2] C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 n-9 *cis* = oleic acid; C18:2 n-6 *cis* = linoleic acid.

fatty acids increased from 26.1 to 39.9% in the corn oil when heated for 24 h and concluded that linoleic acid decreased more rapidly than the total unsaturation.

In order to examine effectiveness of the phenolics throughout storage, the effect of gallic acid and quercetin amount on major fatty acid composition of the mixed oil at 60th and 120th day of storage is presented in Figure 2. Ellagic acid was not included since it is the least effective compound. As can be seen from the figures, generally fatty acid contents decreased with the increasing of gallic acid amount. When the figures were compared, it could be concluded that behaviour of fatty acid change with respect to phenolic compound concentrations changed during storage period. Therefore, consumption period of the oil samples could be determined in order to increase effectiveness of the phenolic compounds used. Multiple response optimisation methodology was performed to optimise phenolic compound concentration at different storage time periods. Percentage change in all fatty acid based on initial fatty acid composition of the oil was calculated. During optimisation, our aim was to minimise change in fatty acid composition. We adjusted criteria of optimisation process by minimising relative percentage the change of the main fatty acids (palmitic, stearic, oleic, and linoleic acid). It is possible but not necessary to model each responses separately in multiple response optimisation in which the modelling is carried out considering all factors

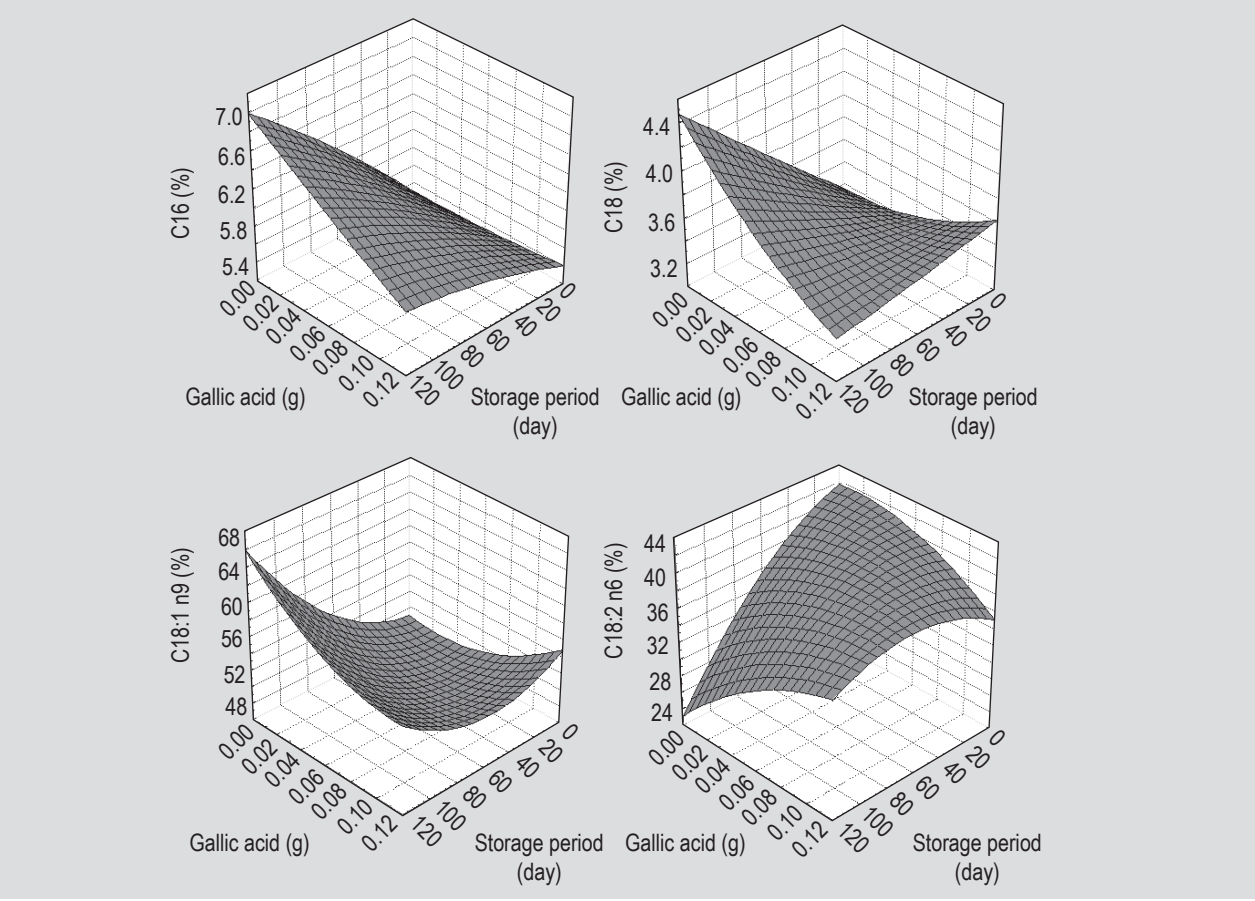

Figure 1. The response surface plots showing the effect of storage period and gallic acid on different fatty acid content (C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 n9 = oleic acid; C18:2 n6 = linoleic acid) of mixed oil stored at 50 °C for 120 days.

simultaneously. Therefore, it is very useful in the food industry. According to the results of the optimisation, optimum concentrations of ellagic, gallic and quercetin in total phenolic amount (0.1 g) were found to be 29.4, 41.2 and 29.4%, respectively, at 60 day of storage; those were found to be 20, 46.7 and 33.3%, for 120 days, respectively. As is seen from the results, distribution of phenolic concentration could change during storage. Application of multiple response optimisation method could eliminate this disadvantage, which is quite necessary for the food industry.

Predictive regression models

Figure 3 shows the regression coefficients and statistically significance levels for linear, interaction and quadratic effects of each major fatty acid. Storage period caused a significant increase in palmitic, stearic and oleic acid and decrease in linoleic acid content of the mixed oil sample (P<0.05) and gallic acid was the most effective one considering the prevention of changes in fatty acid composition, which was followed by quercetin and ellagic acid, respectively. Effective regression equations were constructed for the estimation of major fatty acid content of mixed oil at different storage period and with the addition of phenolic acid at different concentrations. Coefficients of determination were calculated to be 0.735, 0.860, 0.958 and 0.926 for palmitic, stearic, oleic and linoleic acid, respectively.

Lack of fit value for palmitic acid was found to be significant which means that the order of the regression was not secondary (Table 4). However, Box and Drapper (1987) reported that a model with significant lack of fit could still be used when a large amount of data was included in the analysis. It was also reported that the high coefficient of determination value (R^2) is the evidence of the applicability of the regression model between the ranges of variables included (Martínez and Pilosof, 2012). Addition of gallic acid retarded the oxidation significantly (P<0.05) and in general gallic acid and quercetin were found to be effective on the preservation of oil against oxidation. Use of phenolic substance reduced the changes in fatty acid composition of the oil sample due to the developed oxidation. RSM was successfully used to determine the effects of storage period and phenolic compounds on the oxidation parameters of the oil in previous study (Karaman *et al.*, 2014) and major fatty acid composition of mixed oil in the present study. Predictive regression equations having high accuracy were

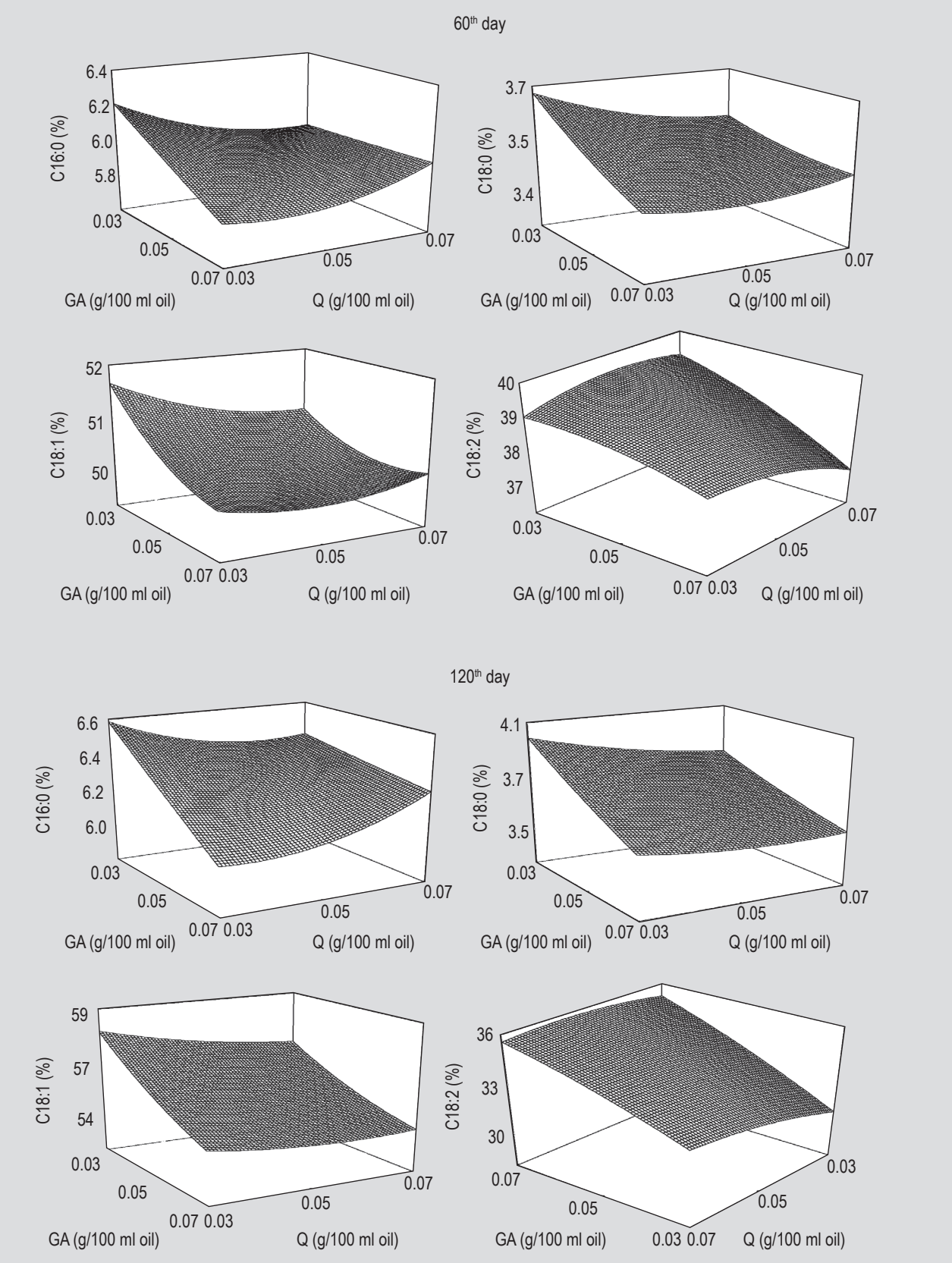

Figure 2. Changes in fatty acid contents with respect to gallic acid (GA) and quercetin amount (Q) at the 60th and 120th day of storage period (C16:0 = palmitic; C18:0 = stearic; C18:1 = oleic; C18:2 = linoleic acid).

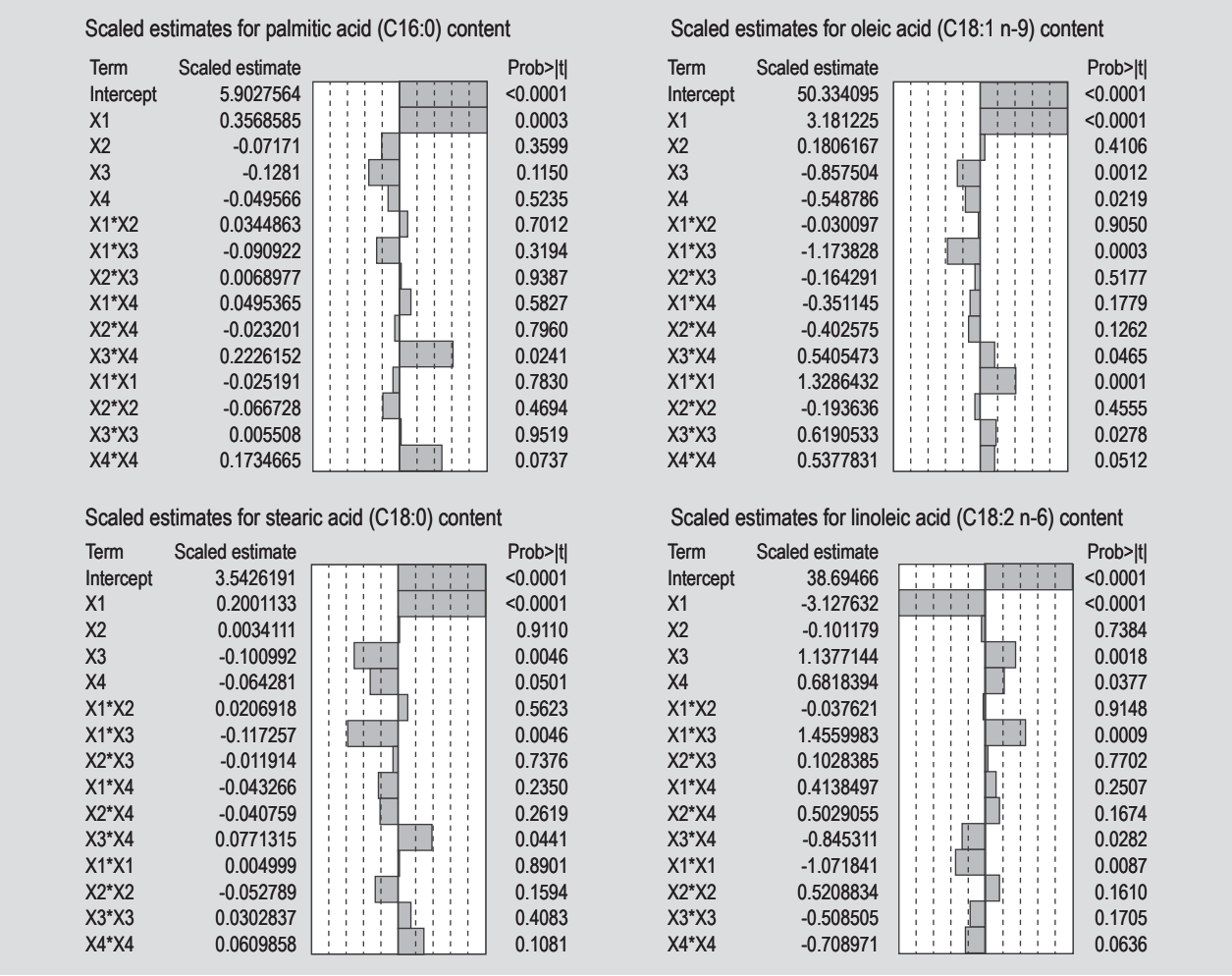

Figure 3 Scaled estimates for different fatty acid contents (C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 n-9 *cis* = oleic acid; C18:2 n-6 *cis* = linoleic acid) showing the direction of linear, interaction and quadratic effects of the processing variables: X1 = storage period (day); X2 = ellagic acid (g); X3 = gallic acid (g); X4 = quercetin (g). Positive and negative scaled estimates values indicate the direction of the increment and decrement, respectively.

constructed for the estimation of each studied parameters with different storage period and phenolic concentration with rather high determination coefficients ($R^2 \geq 0.735$).

4. Conclusions

It was concluded that the fatty acid composition of the edible oils changed significantly during storage because of the oxidation. To retard or prevent the change in fatty acid composition, antioxidants should be used. Phenolic substances like gallic acid, quercetin and ellagic acid could be used to preserve the edible oil quality with preventing the oxidation and change of fatty acid composition. During the storage, an increase in oleic acid level was observed while a decrease in linoleic acid content was determined. Gallic acid was the most effective phenolic compound against oxidation. To minimise the change of fatty acid composition during storage of edible oils, phenolic substance mixture having gallic acid at certain concentration could be used.

Table 4. Significance of the regression models (F-values) and the effects of processing variables on fatty acid content of mixed oil stored at 50 °C for 120 days.

Source[1]	Major fatty acid composition (%)[2]			
	C16:0 (%)	C18:0 (%)	C18:1 n-9 *cis* (%)	C18:2 n-6 *cis* (%)
X_1	22.195***	44.541***	223.22***	110.877***
X_2	0.896	0.013	0.720	0.116
X_3	2.860	11.343***	16.217***	14.670***
X_4	0.428	4.595**	6.642**	5.269**
X_1X_2	0.153	0.352	0.015	0.012
X_1X_3	1.066	11.314***	22.484***	17.776***
X_1X_4	0.006	0.117	0.440	0.089
X_2X_3	0.316	1.540	2.012	1.436
X_2X_4	0.069	1.367	2.645	2.121
X_3X_4	6.389**	4.895**	4.767**	5.991**
X_1X_1	0.079	0.020	27.747***	9.279***
X_2X_2	0.553	2.209	0.589	2.191
X_3X_3	0.004	0.727	6.022**	2.088
X_4X_4	3.736*	2.947	4.545**	4.059*
Model	2.768**	6.142***	22.715***	12.569***
Lack of fit	5.566*	3.315	2.614	3.731
R^2	0.735	0.860	0.958	0.926

[1] X_1 = storage period (day); X_2 = ellagic acid (g); X_3 = gallic acid (g); X_4 = quercetin (g); R^2 = coefficient of determination.
[2] C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 n-9 *cis* = oleic acid; C18:2 n-6 *cis* = linoleic acid.
***P<0.01; **P<0.05; *P<0.1.

References

Ahn, J.H., Kim, Y.P., Seo, E.M., Choi, Y.K. and Kim, H.S., 2008. Antioxidant effect of natural plant extracts on the microencapsulated high oleic sunflower oil. Journal of Food Engineering 84: 327-334.

Armando, C., Maythe, S. and Beatriz, N.P., 1998. Antioxidant activity of grapefruit seed extract on vegetable oils. Journal of the Science of Food and Agriculture 77: 463-467.

Box, G. and Drapper, N., 1987. Empirical model-building and response surfaces. Wiley, New York, NY, USA.

Cook, N.C. and Samman, S., 1996. Flavonoids – chemistry, metabolism, cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry 7: 66-76.

Crapiste, G.H., Brevedan, M.I.V. and Carelli, A.A., 1999. Oxidation of sunflower oil during storage. Journal of American. Oil Chemist's Society 76: 1437-1443.

Erkan, N., Ayranci, G. and Ayranci, E., 2009. A kinetic study of oxidation development in sunflower oil under microwave heating: effect of natural antioxidants. Food Research International 42: 1171-1177.

Goli, A.H., Barzegar, M. and Sahari, M.A., 2005. Antioxidant activity and total phenolic compounds of pistachio (*Pistachia vera*) hull extracts. Food Chemistry 92: 521-525.

Halliwell, B., Murcia, M.A., Chirico, S. and Aruoma, O.I., 1995. Free radicals and antioxidants in food and *in vivo*: what they do and how they work. Critical Reviewes on Food Science and Nutrition 35: 7-20.

Holman, R.T. and Elmer, O.C., 1947. The rates of oxidation of unsaturated fatty acids and esters. Journal American Oil Chemist's Society 24: 127-129.

Johnson, O.C. and Kummerow, F.A., 1957. Chemical changes which take place in an edible oil during thermal oxidation. Journal American Oil Chemist's Society 34: 407-409.

Karaman, S., Toker, O.S., Ozturk, I., Yalcin, H., Kayacier, A., Dogan, M. and Sagdic, O., 2014. A response surface methodology study on the effects of some phenolics and storage period length on vegetable oil quality: change in oxidation stability parameters. Turkish Journal of Agriculture and Forestry 38: 759-772.

Maniak, B. and Targonski, Z., 1996. Przeciwutleniacze naturalnie wystepujace w ywnosci [Antioxidant naturally occur in food]. Przem Ferm Owoc Warz 4: 7-9. [in Polish].

Martínez, K.D. and Pilosof, A.M.R., 2012. Relative viscoelasticity of soy proteinhydrolysate and polysaccharides mixtures at cooling conditions analyzed by response surface methodology. Food Hydrocolloids 26: 318-322.

Matalgyto F,S. and Al-Khalifa, A.S., 1998. Effect of microwave oven heating on stability of some oil and fats. The Arab Gulf Journal of Scientific Research 16: 21-40.

Mohdaly, A.A.A., Sarhan, M.A., Mahmoud, A., Ramadan, M.F. and Smetanska, I., 2010. Antioxidant efficacy of potato peels and sugar beet pulp extracts in vegetable oils protection. Food Chemistry 123: 1019-1026.

Neff, W.E., Mounts, T.L., Rinsch, W.M., Konishi, H. and EI Agaimy, M.A., 1994. Oxidative stability of purified canola oil triacylglycerols with altered fatty acid compositions as affected by triacylglycerol composition and structure. Journal of American Oil Chemist's Society 71: 1101-1109.

Rehman, Z.U., 2006. Citrus peel extract – a natural source of antioxidant. Food Chemistry 99: 450-454.

Rehman, Z.U., Habib, F. and Shah, W.H., 2004. Utilization of potato peel extract as a natural antioxidant in soybean oil. Food Chemistry 85: 215-220.

Ural, A. and Kilic, I., 2006. Data analysis with SPSS. Detay Publishing, Ankara, Turkey.

Wang, L., Wang, G., Zhang, J., Zhang, G., Jia, L., Liu, X., Deng, P. and Fan, K., 2011. Extraction optimization and antioxidant activity of intracellular selenium polysaccharide by *Cordyceps sinensis* SU-02. Carbohydrate Polymers 86: 1745-1750.

Yalcin, H., 2011. Antioxidative effects of some phenolic compounds and carotenoids on refined hazelnut oil. Journal of Consumer Protection and Food Safety 6: 353-358.

Yalcin, H., Karaman, S. and Ozturk, I., 2011. Evaluation of antioxidant efficiency of potato and orange peel and apple pomace extracts in sunflower oil. Italian Journal of Food Science 23: 55-61.

Yalcin, H., Toker, O.S. and Dogan, M., 2012a. Effect of oil type and fatty acid composition on dynamic and steady shear rheology of vegetable oils. Journal of Oleo Science 61: 181-187.

Yalcin, H., Toker, O.S., Ozturk, I., Dogan, M. and Kisi, O., 2012b. Prediction of fatty acid composition of vegetable oils based on rheological measurements using nonlinear models. European Journal of Lipid Science and Technology 114: 1217-1224.

Yuksel, F., Karaman, S. and Kayacier A., 2014. Enrichment of wheat chips with omega-3 fatty acid by flaxseed addition: textural and some physicochemical properties. Food Chemistry 145: 910-917.