Utilization of molecular distillation for determining the effects of some minor compounds on the quality and frying stability of olive pomace oil

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Revised: 7 May 2019 / Accepted: 14 May 2019 / Published online: 10 June 2019
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Abstract The objective of this study was to determine the effects of some minor components such as squalene and mono- and diglycerides (MDG) on the frying stability of olive pomace oil. Refined olive pomace oil was distilled using a falling film type short-path distillation unit at 230 °C under a pressure of 0.02 mbar to remove minor components. Distilled olive pomace oil was introduced with approximately 10,000 mg/kg of squalene and 2.5% of MDG. Fryings were performed 8 times/day at 180 °C for 3 min. Whole frying procedure proceeded for 5 days. Total polar compounds, polymerized triglycerides (PTG), free fatty acids, viscosity, color index, *p*-anisidine value, smoke point, fatty acid composition and iodine values of the oils were monitored. Results indicated that all criteria except smoke point and iodine value increased in all fractions during fryings. The lowest total polar compound was obtained in distilled olive pomace oil while PTG was the lowest in the MDG added fraction. Lower increases in viscosity and color indexes were recorded in squalene incorporated samples. It was observed that squalene and MDG were not effective on the oxidation rate of unsaturated fatty acids during frying.

Keywords Frying stability · Olive pomace oil · Mono- and diglycerides · Squalene · Molecular distillation

Introduction

Deep fat frying which is commonly used for cooking and manufacturing of foods is performed at 150–190 °C (Choe and Min 2007). The oxidative stability of frying oils is also affected by the minor compounds as well as the fatty acid composition (Normand et al. 2001; Rossi et al. 2007). Various minor compounds such as hydrocarbons, sterols, tocopherols, color compounds and trace metals are present in oils and fats, and the majority of these compounds are reported to have positive effects on the frying stability of oils (Kochhar 2001). In contrast, some minor compounds might have detrimental effects on oil stability during deep frying. Free fatty acids (FFAs) catalyze further hydrolysis of triglycerides and promote oxidation. Lecithins can cause darkening and foaming which may also stem from mono- and diglycerides (MDG) (Kochhar 2001).

Olive pomace oil is obtained from the dry olive cake by solvent extraction. Therefore, the crude oil contains a high amount of minor compounds such as sterols, MDG and squalene. Refined olive pomace oil is also rich in these substances. Diglycerides cannot be fully removed from the oil during refining. Besides, a considerable amount of squalene retains in the oil.

Molecular distillation has been accepted as an efficient way of separating heat-sensitive compounds at reduced evaporation temperatures due to the applied vacuum inside the distillation chamber. In this system, a film layer of the liquid mixture is formed and the liquid molecules evaporate at low temperatures without any negative effects of heat. This technique has been used in many industries such

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as pharmaceutical and petrochemical for many years, but its use in the food industry has accelerated in recent years. Molecular distillation enables an effective separation of many commercially valuable compounds, toxic substances, contaminants/pollutants, and healthful or therapeutical compounds from the food materials. For instances, separation of squalene—a long-chained hydrocarbon (Babeau et al. 2018; Ketenoglu et al. 2018), essential oil processing (Mezza et al. 2018; Borgarello et al. 2015; Ruben et al. 2014), concentration of omega fatty acids (Zhang et al. 2013), removal of organic pollutants (Olli et al. 2013; Oterhals et al. 2010) and also bio-oil processing and production (Wang et al. 2009) can be achieved by molecular distillation.

The objective of this study was to investigate the individual effects of partial glycerides and squalene on the frying stability of olive pomace oil. For this aim, oil fractions having different minor composition were prepared and the physical and chemical changes of the oils were monitored during frying.

Materials and methods

Materials

Refined olive pomace oil was purchased from Bilginoğlu Marbil Yağ San. Tic. A.Ş. (İzmir, Turkey). Frozen crispy potato sticks (7 mm × 7 mm) (SuperFresh Kerevitaş Gıda San. Tic. A.Ş., Turkey) were purchased from a local market. All reagents were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). The MDG mixture was prepared according to the method described previously by Nouredini et al. (2004).

Short-path distillation

A KDL5 short-path distillation unit (UIC GmbH, Alzenau-Hoerstein, Germany) was used to remove primary minor compounds (squalene, MDG, FFA). The operating conditions were as follows:

- 0.02 mbar operating pressure
- 230 °C evaporation temperature
- 3 mL/min feeding rate
- 240 rpm wiper rolling speed
- 0.048 m² evaporation surface area
- 20 °C condenser temperature

The distillate was named as the 1st fraction containing 0.08% FFAs, 126.98 mg/kg squalene and 0.24% MDG.

Preparation of oil fractions

Squalene and MDG were introduced to the 1st fraction at different proportions in order to produce 2nd and 3rd fractions. The 2nd fraction was prepared by the addition of approximately 10,000 mg/kg of squalene to the 1st fraction. The 3rd fraction was prepared by addition of 2.5% of MDG to the 1st fraction.

Deep fat frying

Frying treatments were performed 8 times in a day at 180 °C. The frying time was 3 min in every one hour. A double-chambered fryer (Model GN 1/2, Öztiryakiler, Turkey) was used. Totally 40 frying treatments were carried out during 5 days. 3.75 L of frying media and 200 g of potatoes were used in every frying and 100 mL of oil samples were taken after the 4th and the 8th fryings. The samples were filtered and stored at − 18 °C until analyzed. The necessary amount of oil was added to compensate the absorbed oil prior to fryings on each day.

Chemical and physical analyses

Squalene

Squalene contents of oils were determined according to AOCS Official Method Ch 8–02 (AOCS 2003). A gas chromatograph (GC 2010, Shimadzu, Japan) equipped with an HP-5 fused silica capillary column (12 m × 0.32 mm i.d. × 0.25 µm film thickness) (Agilent J&W, CA, USA) was used for this analysis. Helium was the carrier gas with a flow rate of 1.8 mL/min in splitless mode. Flame ionization detector (FID) and injector temperatures were 350 and 300 °C, respectively. Column temperature programme was as follows:

- Start with 80 °C and hold for 1 min
- Ramp to 240 °C; rate: 20 °C/min
- Ramp to 325 °C; rate: 5 °C/min and hold at this temperature for 6 min
- Ramp to 340 °C; rate: 20 °C/min and hold at this temperature for 10 min

The total duration of the GC method was 42.75 min.

MDG content

MDG contents of oils were determined according to AOCS Official Method Cd 11d-96 (AOCS 2003). A Prominence LC-20A HPLC system (Shimadzu, Japan) equipped with an evaporative light scattering detector (Sedex 80LT, Sedere, France) and a Lichrosorb Si60-5 column (250 mm × 4.6 mm i.d. × 5 µm particle size) (Hichrom,

Table 1 Mobile phase flow chart used in HPLC

Time (min)	Flow rate (mL/min)	A (%)	B (%)
0	2	98	2
13.4	2	65	35
14.2	2	2	98
25	2	2	98
25.2	2	98	2
32	2	98	2

Reading, UK) was used. The column temperature was 40 °C. The flow rate was 2 mL/min with the following gradient programme:

- Mobile phase contains: Hexane (A); Hexane/2-propanol/ethyl acetate/(10%, v/v) formic acid (80:10:10:1, v/v/v/v) (B)
- Mobile phase chart is presented in Table 1.

Total polar compounds

Total polar compounds were measured with a food oil monitor (FOM 310, Ebro, Germany) during frying. The probe was immersed into the hot oil (approx. 180 °C) after 20 min following the end of the frying, aiming to allow small food particles to settle which were possibly present in the frying oil. The instrument was switched on while gently stirring the oil for uniform measurement and data were collected after 2–3 s.

Polymerized triglycerides

Polymerized triglyceride contents (PTG) of the oils were determined according to AOCS Official Method Cd 22–91 (AOCS 2003). 0.2 g of oil sample was dissolved in 15 mL of tetrahydrofuran and the mixture was then injected to a Prominence LC-20A HPLC (Shimadzu, Japan) equipped with a Phenogel guard column (50 × 7.8 mm, 5 µm particle size, Phenomenex, Torrance, CA) followed by two connected Phenogel columns -one having a pore size of 500 Å and the other having 100 Å—(300 × 7.8 mm, 5 µm particle size each) (Phenomenex, Torrance, CA). An evaporative light scattering detector (Sedex 80LT, Sedere, France) was used with the mobile phase as tetrahydrofuran having a flow rate of 1 mL/min.

Viscosity

Viscosities of the oils were measured at 20 °C under a shear rate of 10 s⁻¹ using an AR2000 EX rheometer (TA Instruments, New Castle, DE). An acrylic probe having a

diameter of 40 mm was used and results were expressed in mPa.s.

Color index, FFA content, *p*-anisidine value and smoke point

Color indexes, FFA contents, *p*-anisidine values and smoke points were determined according to AOCS Official Methods Cc 13c-50, Ca 5a-40, Cd 18-90, Cc 9a-48, respectively (AOCS 2003). A U-2800 model spectrophotometer (Hitachi, Tokyo, Japan) was used for the relevant spectrophotometric analyses.

Fatty acid composition and iodine value

The methyl esters of the fatty acids were prepared according to IUPAC (1987) and analyzed using a GC-2010 gas chromatograph (Shimadzu, Japan) equipped with a DB-23 column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Agilent J&W, CA, USA). Injector, oven and detector temperatures were 230, 190 and 240 °C, respectively. The split ratio was 1:80. Helium was used as the carrier gas with a flow rate of 0.5 mL/min. Iodine values were calculated using the formula in the AOCS Official Method Cd 1c-85 (AOCS 2003).

Statistical analysis

All data were obtained from duplicates and reported as mean ± standard deviation. Differences of means were statistically analyzed by two-way analysis of variance (ANOVA) and Duncan's multiple range test. Significances of frying time, oil fractions and their interactions were compared with a confidence level of 95%. Statistical analyses were carried out with Minitab 16 (Minitab Inc., State College, PA, USA).

Results and discussion

Results of analyzed fractions prior to fryings

Refined olive pomace oil containing 0.17% of FFA, 6831.25 mg/kg of squalene and 1.29% of MDG was distilled using a short-path distillation unit at 0.02 mbar and 230 °C. After distillation FFA, squalene and MDG contents in distilled olive pomace oil (1st fraction) were reduced to 0.08%, 126.98 mg/kg and 0.24%, respectively. The 1st fraction was then incorporated by squalene and MDG in order to investigate their individual effects on the frying stability of olive pomace oil. Physical and chemical characteristics of the distilled and minor component added oils are presented in Table 2. The 2nd fraction which was

Table 2 Physical and chemical characteristics of oil fractions

	1st fraction (Distilled oil)	2nd fraction (Squalene)	3rd fraction (MDG)
Free fatty acids, FFA (% oleic acid)	0.08 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Squalene (mg/kg)	126.98 ± 2.47	10,466.9 ± 120.03	126.98 ± 2.47
Mono and di-glycerides, MDG (%)	0.24 ± 0.01	0.24 ± 0.01	2.55 ± 0.06
Total polar compounds (%)	8.50 ± 0.71	10.00 ± 0.01	21.00 ± 0.71
Polymerized triglycerides, PTG (%)	0.03 ± 0.01	0.04 ± 0.01	0.01 ± 0.01
Viscosity (mPa s)	94.0 ± 0.01	92.0 ± 0.01	94.0 ± 0.01
Color index	4.34 ± 0.01	3.57 ± 0.01	3.91 ± 0.01
<i>p</i> -anisidine value	6.66 ± 0.01	7.12 ± 0.01	7.43 ± 0.01
Smoke point (°C)	242 ± 0.03	202 ± 0.03	194 ± 0.03

Values are mean ± standard deviation of duplicates

prepared by the addition of squalene to the 1st fraction contained 10,466.9 mg/kg squalene. MDG content of the 3rd fraction which was prepared by addition 2.5% of partial glycerides was determined as 2.55%. The amount of PTG content was nearly zero, while total polar compounds content ranged between 8.5–21.0%. Smoke point was the highest in the 1st fraction (242 °C); however, it was the lowest in the 3rd fraction (194 °C) containing MDG. Squalene addition also reduced the initial smoke point from 242 to 202 °C.

Results of analyzed fractions during fryings

All results belonging to physicochemical properties of samples which were periodically taken during fryings were tabulated in Table 3. Also, the results were discussed in detail at the following subsections separately.

Total polar compounds and polymerized triglycerides

The changes in the polar compound and PTG contents during 40 fryings for 5 days are visualized in Fig. 1a, b, respectively. Polar compound contents increased with the subsequent fryings, as indicated by another study (Houhoula et al. 2003). It was initially the lowest in the 1st fraction as 8.5% and increased up to 24.75% at the end of the 40th frying. The 1st fraction was the only one having polar compounds below the legal limit of 25% at the end of 40th frying, which suggested that polar compound contents of the fresh oils determine the final quality of the oils used for frying. Polar compound content in the 2nd fraction after the 40th frying was slightly higher than 25% (27.75%); however, polar compounds of the 3rd fraction reached to 38.5% after the 40th frying due to its considerably higher initial polar

compound content as 21.0%. These results indicated that increases in the polar compound during frying were determined by initial polar compound content of the oil. Squalene is a hydrocarbon and did not increase initial polar content significantly, while MDGs are polar compounds and considerably increased both initial and final polar compound content.

PTG content increased during fryings for each fraction. All fractions had almost the same PTG values prior to frying treatments. The lowest increase was determined in the 3rd fraction containing more MDG while the other fractions had very similar PTG values after subsequent fryings. The PTG content of the 3rd fraction increased from 0.01 to 3.01% at the end of fryings. Some researchers used sunflower oil for 75 repeated fryings with a fast turnover of fresh oil and found that polymers increased from 0.1 to 1.65% and 3.44% at 20th and 75th fryings, respectively (Cuesta et al. 1993). In another study, it was reported that the PTG content was lower for diacylglycerol-rich oil than triacylglycerol oil (Shimizu et al. 2004). These results suggested that PTG formation might not be a primary reaction for diacylglycerols during frying since the 3rd fraction was oxidized more rapidly than the others.

Viscosity

Figure 2a shows the changes in viscosity values of the fractions. Similar viscosity values were determined for the 1st and 3rd fractions throughout 40 fryings even the 3rd fraction with higher MDG generated lower PTG. However, squalene utilization in the 2nd fraction resulted in the lowest viscosity increments. The viscosity of the 2nd fraction increased from 92.0 mPa s to 118.50 mPa.s after 40th frying. There are some studies in the literature reporting increases in viscosity of the oils during frying (Kalogianni et al. 2011; Tseng et al. 1996).

Table 3 Physicochemical characteristics of fractions during fryings

Frying time (h)	Fractions (nth)	Physicochemical Analyses						
		Total polar compounds (%)	Total polymer triglycerides (%)	Viscosity (mPa s)	Color index	FFA (%)	p-anisidine value	Smoke point (°C)
0	1	8.50 ± 0.71 ^{Cj}	0.03 ± 0.01 ^{Ai}	94.00 ± 0.01 ^{Ah}	4.34 ± 0.01 ^{Af}	0.08 ± 0.01 ^{Ae}	6.66 ± 0.01 ^{Ah}	242 ± 0.03 ^{Aa}
	2	10.00 ± 0.01 ^{Bk}	0.04 ± 0.01 ^{Ai}	92.00 ± 0.01 ^{Ag}	3.57 ± 0.01 ^{Aa}	0.02 ± 0.01 ^{Bk}	7.12 ± 0.01 ^{Ag}	202 ± 0.03 ^{Ba}
	3	21.00 ± 0.71 ^{Aj}	0.01 ± 0.01 ^{Ah}	94.00 ± 0.01 ^{Ai}	3.91 ± 0.01 ^{Ah}	0.03 ± 0.01 ^{Bi}	7.43 ± 0.01 ^{Ah}	194 ± 0.03 ^{Ca}
4	1	10.75 ± 0.35 ^{Ci}	0.46 ± 0.19 ^{Ah}	94.50 ± 0.71 ^{Ah}	5.01 ± 0.34 ^{Bef}	0.08 ± 0.01 ^{Be}	29.92 ± 4.61 ^{Bg}	234 ± 1.41 ^{Ab}
	2	12.25 ± 0.35 ^{Bj}	0.38 ± 0.02 ^{Ah}	93.00 ± 0.00 ^{Ag}	4.35 ± 0.01 ^{Ba}	0.05 ± 0.00 ^{Cj}	34.40 ± 0.93 ^{Af}	196 ± 1.41 ^{Bb}
	3	23.25 ± 0.35 ^{Ai}	0.14 ± 0.00 ^{Bh}	94.25 ± 0.35 ^{Ai}	6.60 ± 0.13 ^{Ag}	0.17 ± 0.02 ^{Ah}	26.99 ± 1.39 ^{Bg}	191 ± 0.71 ^{Cb}
8	1	12.50 ± 0.71 ^{Ch}	1.21 ± 0.25 ^{Ag}	97.75 ± 1.77 ^{Ag}	6.16 ± 0.10 ^{ABde}	0.09 ± 0.01 ^{Be}	42.84 ± 5.61 ^{Bf}	227 ± 0.71 ^{Ac}
	2	14.50 ± 0.00 ^{Bi}	0.87 ± 0.08 ^{Bg}	96.00 ± 0.00 ^{Af}	5.11 ± 0.32 ^{Ba}	0.09 ± 0.01 ^{Bi}	47.50 ± 1.21 ^{Ae}	192 ± 0.71 ^{Bc}
	3	25.50 ± 0.71 ^{Ah}	0.42 ± 0.01 ^{Cg}	98.00 ± 0.00 ^{Ah}	7.34 ± 0.17 ^{Ag}	0.25 ± 0.02 ^{Ag}	40.64 ± 1.08 ^{Bf}	184 ± 0.71 ^{Cc}
12	1	14.50 ± 0.71 ^{Cg}	1.46 ± 0.01 ^{Af}	102.75 ± 2.47 ^{Af}	6.28 ± 0.78 ^{Bde}	0.19 ± 0.01 ^{Bd}	48.94 ± 2.36 ^{Be}	217 ± 0.00 ^{Ad}
	2	16.75 ± 0.35 ^{Bh}	1.39 ± 0.09 ^{Af}	97.75 ± 1.06 ^{Bf}	5.00 ± 0.01 ^{Ba}	0.15 ± 0.01 ^{Ch}	54.16 ± 1.31 ^{Ad}	192 ± 0.71 ^{Bc}
	3	28.25 ± 0.35 ^{Ag}	0.74 ± 0.03 ^{Bf}	102.50 ± 0.71 ^{Ag}	9.23 ± 0.29 ^{Af}	0.30 ± 0.03 ^{Af}	51.26 ± 1.35 ^{ABe}	183 ± 3.54 ^{Cc}
16	1	16.00 ± 0.71 ^{Cf}	1.83 ± 0.30 ^{Ae}	109.50 ± 1.41 ^{Ae}	6.58 ± 0.92 ^{Bde}	0.21 ± 0.03 ^{Bd}	55.63 ± 2.93 ^{Bd}	213 ± 2.12 ^{Ae}
	2	18.50 ± 0.71 ^{Bg}	1.85 ± 0.03 ^{Ae}	100.75 ± 1.06 ^{Ce}	4.48 ± 0.01 ^{Ca}	0.19 ± 0.02 ^{Bg}	60.07 ± 0.46 ^{Ac}	190 ± 0.00 ^{Bcd}
	3	30.00 ± 0.00 ^{Af}	1.30 ± 0.01 ^{Be}	106.50 ± 0.71 ^{Bf}	9.41 ± 0.16 ^{Af}	0.37 ± 0.02 ^{Ae}	61.88 ± 1.11 ^{Ad}	178 ± 2.12 ^{Cd}
20	1	17.50 ± 0.71 ^{Ce}	2.13 ± 0.23 ^{Ad}	112.50 ± 0.71 ^{Ad}	7.13 ± 1.54 ^{Bcd}	0.24 ± 0.01 ^{Bc}	59.98 ± 0.06 ^{Bc}	209 ± 1.41 ^{Af}
	2	20.00 ± 0.00 ^{Bf}	2.07 ± 0.14 ^{Ae}	103.25 ± 0.35 ^{Cd}	4.14 ± 0.05 ^{Ca}	0.25 ± 0.01 ^{Bf}	64.62 ± 1.66 ^{Ab}	188 ± 0.71 ^{Bde}
	3	32.00 ± 0.71 ^{Ae}	1.55 ± 0.00 ^{Bd}	110.00 ± 0.71 ^{Be}	10.65 ± 0.20 ^{Aef}	0.38 ± 0.01 ^{Ae}	63.09 ± 0.56 ^{ABd}	179 ± 0.00 ^{Cd}
24	1	19.50 ± 0.71 ^{Cd}	2.77 ± 0.17 ^{Ac}	118.00 ± 1.41 ^{Ac}	7.57 ± 1.28 ^{Bcd}	0.26 ± 0.02 ^{Bc}	64.32 ± 1.09 ^{Bab}	206 ± 2.12 ^{Ag}
	2	21.50 ± 0.00 ^{Be}	2.62 ± 0.01 ^{Ad}	106.50 ± 0.00 ^{Cc}	4.53 ± 0.05 ^{Ca}	0.28 ± 0.02 ^{Be}	67.58 ± 1.20 ^{ABab}	187 ± 0.71 ^{Be}
	3	33.25 ± 0.35 ^{Ad}	1.70 ± 0.02 ^{Bd}	114.25 ± 1.06 ^{Bd}	11.48 ± 0.24 ^{Ad}	0.44 ± 0.01 ^{Ad}	70.36 ± 0.34 ^{Ac}	175 ± 0.71 ^{Ce}
28	1	20.50 ± 0.71 ^{Cc}	2.84 ± 0.12 ^{Ae}	119.25 ± 3.18 ^{Ac}	7.98 ± 1.41 ^{Bbc}	0.26 ± 0.03 ^{Cc}	60.35 ± 3.83 ^{Cc}	199 ± 0.71 ^{Ah}
	2	23.00 ± 0.00 ^{Bd}	2.87 ± 0.10 ^{Ac}	106.75 ± 0.35 ^{Cc}	4.73 ± 0.04 ^{Ca}	0.33 ± 0.00 ^{Bd}	66.57 ± 0.22 ^{Bab}	189 ± 0.00 ^{Bde}
	3	34.75 ± 0.35 ^{Ac}	2.15 ± 0.02 ^{Bc}	116.50 ± 0.71 ^{Bc}	12.95 ± 0.71 ^{Ad}	0.48 ± 0.02 ^{Ac}	70.56 ± 0.84 ^{Ac}	175 ± 1.41 ^{Ce}
32	1	22.00 ± 0.71 ^{Cb}	3.29 ± 0.30 ^{Ab}	124.00 ± 4.24 ^{Ab}	9.23 ± 1.91 ^{Bab}	0.29 ± 0.02 ^{Cb}	61.49 ± 0.59 ^{Cbc}	195 ± 0.71 ^{Ai}
	2	24.75 ± 0.35 ^{Bc}	3.30 ± 0.00 ^{Ab}	112.25 ± 0.35 ^{Cb}	4.72 ± 0.05 ^{Ca}	0.42 ± 0.00 ^{Bc}	69.65 ± 0.19 ^{Ba}	188 ± 0.71 ^{Bde}
	3	36.50 ± 0.00 ^{Ab}	2.61 ± 0.04 ^{Bb}	121.75 ± 0.35 ^{Bb}	14.04 ± 1.04 ^{Ac}	0.52 ± 0.00 ^{Ab}	77.76 ± 0.45 ^{Ab}	174 ± 0.71 ^{Ce}
36	1	22.50 ± 0.71 ^{Cb}	3.40 ± 0.31 ^{Ab}	124.00 ± 1.41 ^{Ab}	9.26 ± 2.24 ^{Bab}	0.35 ± 0.00 ^{Ca}	61.59 ± 0.29 ^{Cbc}	196 ± 0.71 ^{Ai}
	2	25.75 ± 0.35 ^{Bb}	3.38 ± 0.24 ^{Ab}	113.25 ± 1.77 ^{Bb}	4.25 ± 0.05 ^{Ca}	0.46 ± 0.02 ^{Bb}	67.92 ± 1.08 ^{Bab}	182 ± 2.12 ^{Bf}
	3	36.75 ± 0.35 ^{Ab}	2.75 ± 0.02 ^{Bb}	123.25 ± 1.06 ^{Ab}	16.11 ± 1.40 ^{Ab}	0.53 ± 0.00 ^{Ab}	76.67 ± 2.66 ^{Ab}	174 ± 0.71 ^{Ce}

Table 3 continued

Frying time (h)	Fractions (nth)	Physicochemical Analyses						
		Total polar compounds (%)	Total polymer triglycerides (%)	Viscosity (mPa s)	Color index	FFA (%)	p-anisidine value	Smoke point (°C)
40	1	24.75 ± 1.06 ^{Ca}	3.90 ± 0.10 ^{Aa}	128.25 ± 1.77 ^{Aa}	10.04 ± 2.51 ^{Ba}	0.37 ± 0.02 ^{Ca}	67.09 ± 1.82 ^{Ba}	194 ± 0.71 ^{Al}
	2	27.75 ± 0.35 ^{Ba}	3.97 ± 0.13 ^{Aa}	118.50 ± 0.71 ^{Ba}	4.77 ± 0.01 ^{Ca}	0.53 ± 0.01 ^{Ba}	68.07 ± 2.13 ^{Bab}	179 ± 0.71 ^{Bg}
	3	38.50 ± 0.71 ^{Aa}	3.01 ± 0.02 ^{Ba}	129.25 ± 1.77 ^{Aa}	18.86 ± 1.51 ^{Aa}	0.57 ± 0.01 ^{Aa}	82.98 ± 0.79 ^{Aa}	173 ± 0.71 ^{Ce}

Values are mean ± standard deviation of duplicates

For each analysis individually:

Uppercase letters refer to the differences of means between fractions (1, 2, 3) in the same frying time ($p < 0.05$)

Lowercase letters refer to the differences of means between frying times (0, 4, 8,...,40) in the same fraction ($p < 0.05$)

Color index

The color index can be used to determine the quality of frying oils. The color of the oil is affected by both fatty acids, dimers, polymers, other minor compounds and frying time (Xu et al. 1999). As shown in Fig. 2b, color indexes of the 1st and 3rd fractions generally increased during fryings. Considerable increments in color indexes were determined for the 3rd fraction containing MDG with the subsequent fryings. Initial color index of the 3rd fraction was 3.91 while it increased to 18.86 after 40th frying. However, little changes were obtained for the 2nd fraction containing squalene. Initial and final color indexes of the 2nd fraction were 3.57 and 4.77, respectively.

FFA content

Changes in FFA contents of all fractions during frying treatments are visualized in Fig. 3a. FFA contents of all fractions tended to increase with ongoing fryings; however, the lowest increment was observed in the 1st fraction. FFA percentages of the 1st fraction increased from 0.08 to 0.37% after the 40th frying. However, the highest increase in FFAs was recorded in the 3rd fraction having a final FFA content of 0.57%. This fraction had the highest MDG concentration among all samples, which possibly caused the formation of more FFA during frying. It was reported that the hydrolysis was accelerated by the presence of mono- and diglycerides (Frega et al. 1999). In another study, it was revealed that the increment of the acid value of diacylglycerol-rich oil was higher than that of triacylglycerol-rich oil during frying at 180 °C (Li et al. 2005).

p-anisidine value

p-anisidine forming as a result of the degradation of oils demonstrates the amount of non-volatile aldehydes (principally 2-alkenals and 2,4-dienals). The changes in *p*-anisidine values during frying treatments are presented in Fig. 3b. From the figure, *p*-anisidine values increased for each fraction, while initial *p*-anisidine values for all fractions were very close to each other. After 28th frying, it began to increase for the 3rd fraction and reached to the highest value (82.98) at the end of the frying treatment, which might be resulted in higher FFA content generated by 3rd fraction during frying because it is well known that FFAs are oxidized easier than esterified fatty acids. This fraction also had the highest polar content but the lowest PTG in overall fryings. *p*-anisidine value has been reported to increase with frying time (Tompkins and Perkins 1999). According to another study, it increased within the first 30 h of a total frying period of 72 h. However, this

Fig. 1 **a** Changes in total polar content of the fractions during frying, **b** changes in PTG content of the fractions during frying

increment depended on the oil type. After 30 h, a significant increase was not observed (Matthaus 2006).

Smoke point

At the beginning of frying, smoke points of the 1st, 2nd and 3rd fractions were 242 °C, 202 °C and 194 °C,

respectively. The highest smoke point was observed in the 1st fraction, which was the distilled oil containing very little amount of the minor compounds. Initial smoke points of the other fractions were considerably lower than that of the 1st fraction. As indicated in Fig. 4a, smoke points of the oils decreased during 40 fryings. At the end of the frying, smoke points were determined in the 1st, 2nd and

Fig. 2 **a** Changes in viscosity of the fractions during frying, **b** changes in color index value of the fractions during frying

3rd fractions as 194 °C, 179 °C and 173 °C, respectively. While the highest initial smoke point means the highest final smoke point, the highest decrease was also recorded for the 1st fraction ($\Delta T = 48$ °C). On the other hand, final smoke points of all fractions were higher than 170 °C, which is regarded as a legal limit for frying oils in some

countries. In a previous study, researchers fried potato chips with refined, bleached and deodorized palm olein, soybean oil and their blends at 180 °C for 5 h per day for 5 consecutive days and observed that smoke points of all oils were higher than 170 °C at the end (Man et al. 1999). The results showed that some minor constituents might

Fig. 3 **a** Changes in FFA content of the fractions during frying, **b** changes in *p*-anisidine value of the fractions during frying

reduce smoke points of fresh oil and the highest smoke points might be observed in well-refined vegetable oils. This phenomenon agrees with the negative correlation between FFA and smoke point as previously stated (Kochhar 2001).

Fatty acid composition and IV

The principal factor to determine the oxidation rate throughout the frying process is the fatty acid composition (Rudzińska et al. 2005). The initial and final fatty acid

Fig. 4 **a** Changes in smoke point of the fractions during frying, **b** changes in unsaturated fatty acids of the fractions during frying. UFA / PA: Unsaturated fatty acids/Palmitic acid

compositions and iodine values (IV) of all fractions are given in Table 4. Percentages of oleic (*C18:1*), linoleic (*C18:2*) and linolenic (*C18:3*) acids decreased while palmitic (*C16:0*) and stearic (*C18:0*) acid contents relatively increased during 40 fryings in all fractions. Similarly, Man et al. (1999) reported that palmitic and stearic acids

increased while oleic and linoleic acids decreased after 5 days of intermittent frying at 180 °C.

Decomposition rates of unsaturated fatty acids (oleic, linoleic and linolenic acids) for 1st, 2nd and 3rd fraction were calculated as reduction ratio to initial concentrations. For oleic acid, they were determined as 3.10%, 2.51% and 2.67% for 1st, 2nd and 3rd fraction, while these ratios were

Table 4 Fatty acid compositions (%) and iodine values (IV) of fractions

Fatty acids	1st fraction (Distilled oil)		2nd fraction (Squalene)		3rd fraction (MDG)	
	0th hour	40th hour	0th hour	40th hour	0th hour	40th hour
<i>C14:0</i>	0.01 ± 0.01	0.09 ± 0.01	0.01 ± 0.01	0.08 ± 0.01	0.01 ± 0.01	0.07 ± 0.01
<i>C16:0</i>	10.88 ± 0.01	14.18 ± 0.10	10.83 ± 0.01	13.92 ± 0.11	10.48 ± 0.01	13.58 ± 0.16
<i>C16:1</i>	0.79 ± 0.01	0.73 ± 0.01	0.77 ± 0.01	0.74 ± 0.03	0.93 ± 0.01	0.95 ± 0.10
<i>C17:0</i>	0.09 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
<i>C17:1</i>	0.14 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
<i>C18:0</i>	2.52 ± 0.01	2.98 ± 0.01	2.62 ± 0.01	2.95 ± 0.01	2.37 ± 0.01	2.73 ± 0.11
<i>C18:1</i>	73.55 ± 0.01	71.27 ± 0.03	73.45 ± 0.01	71.61 ± 0.07	74.08 ± 0.01	72.10 ± 0.10
<i>C18:2</i>	10.26 ± 0.01	8.32 ± 0.11	10.35 ± 0.01	8.32 ± 0.04	10.30 ± 0.01	8.28 ± 0.04
<i>C18:3</i>	0.70 ± 0.01	0.42 ± 0.01	0.73 ± 0.01	0.43 ± 0.01	0.66 ± 0.01	0.38 ± 0.01
<i>C20:0</i>	0.47 ± 0.01	0.51 ± 0.01	0.47 ± 0.01	0.50 ± 0.01	0.43 ± 0.01	0.48 ± 0.01
<i>C20:1</i>	0.35 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.35 ± 0.03
<i>C22:0</i>	0.14 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.01
<i>C24:0</i>	0.11 ± 0.01	0.79 ± 0.01	0.09 ± 0.01	0.75 ± 0.01	0.08 ± 0.01	0.74 ± 0.01
IV	87.64 ± 0.01	81.25 ± 0.20	87.77 ± 0.01	81.59 ± 0.14	88.19 ± 0.01	82.05 ± 0.25

Values are mean ± standard deviation of duplicates

18.91%, 19.61% and 19.61% for linoleic acid, respectively. The reductions in linoleic acid were almost 7–8 times higher than those of oleic acid. Decreases in linolenic acid for all fractions were calculated about 40% at the end. The reductions in each fatty acids were very similar for all fractions, which suggests that the oxidation rate of fatty acids during frying was determined mainly by unsaturation degree of them rather than minor compounds such as squalene and MDG. Percentage of palmitic acid, which is primary saturated fatty acid of the olive pomace oil, relatively increased during frying. After the 40th frying, increases in the palmitic acid ratio in the 1st, 2nd and 3rd fractions were calculated as 30.33%, 28.53% and 29.58%, respectively. Changes in C18:1/C16:0 and C18:2/C16:0 ratios for all fractions during frying are given in Fig. 4b. These ratios decreased throughout frying in all oils, but they were similar for all fractions. It was reported that C18:2/C16:0 declined from 2.39 to 2.03 when cottonseed oil was heated at 185 °C for 12 h (Houhoula et al. 2002). Aladedunye and Przybylski (2009) performed frying experiments with canola oil at 185 and 215 °C for 49 h and reported that C18:2/C16:0 reduced from 4.73 to 3.87 and 4.28 to 3.23 at 185 and 215 °C, respectively.

Changes in iodine values (IV) of the oil fractions are also shown in Table 4. IV is used to determine the unsaturation degree of oils. It has been reported that IV decreases due to the degradation of the double bonds in oxidation reactions, particularly in deep fat frying (Alireza et al. 2010). According to Table 4, the IVs of all samples decreased at the end of 40 fryings. Decreases in IV were almost the same in the 1st, 2nd and 3rd fractions (6.39, 6.18 and 6.14, respectively) at the end of frying treatments,

which suggested that squalene and MDG were not effective on the reduction of IV during frying.

Conclusion

Squalene and MDG affected the frying performance of olive pomace oil in both positive and negative manners. Initial polar content, *p*-anisidine value and color index of the oil increased with the presence of MDG during frying treatments while lower PTG formation was observed. Positive effects of squalene were observed on the color index and viscosity. The oxidation rates of fatty acids mainly depended on their unsaturation degrees rather than such minor compounds as squalene and MDG. These results are expected to do the guidance for further research having a similar aim with this study.

Acknowledgements The authors thank the Turkish Scientific Council TUBITAK (TOVAG-Project No: 1100193) for providing funding for the project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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