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The impact of saturated monoacylglycerols on the oxidative stability of Canola oil under various time/temperature conditions

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SUMMARY: Due to the inconsistency of monoacylglycerols' (MAGs) impacts and the lack of research concerning the weight of saturated monoacylglycerols on the oxidative stability of oils, the current study was designed. For this purpose, saturated MAGs at 0.5, 3.0 and 5.0% were added to canola oil and subsequently exposed to a Schaall oven test at 60 °C for 31 days (to assimilate moderate thermal conditions and a prolonged treatment time) and a Rancimat test at 110 °C (to assimilate extreme thermal conditions and shorter treatment time). To evaluate the quality and oxidative stability parameters of MAG-containing canola oil, free fatty acids (FFA), peroxide value (PV), and the oxidative stability index (OSI) were determined. The findings indicated that with the increase in MAG levels, the FFA increased from 0.05 up to 0.2%. The PV increased from 2 to 100 meq/L with the increase in MAG concentration. Also, it was shown that OSI increased from 12.20 to 13.10 h, which was proportional to MAG concentration.

KEYWORDS: *Canola oil; Monoacylglycerols; Oxidative stability; Rancimat test; Schaal oven test*

RESUMEN: ***Influencia de los monoacilgliceroles saturados sobre la estabilidad oxidativa de aceites de canola en diversas condiciones de tiempo / temperatura.*** Dada la discrepancia sobre la influencia de monoacilgliceroles (MAG) y la falta de documentación sobre el peso de los MAG saturados sobre la estabilidad oxidativa de los aceites, se estableció este estudio. Para este propósito, MAG saturados al 0,5, 3,0 y 5,0% fueron agregados a aceites de canola y posteriormente expuestos a la prueba del horno Schaall a 60 °C durante 31 días (simulación de condiciones térmicas moderadas y largo tiempo) y prueba de Rancimat a 110 °C (simulación de condiciones térmicas extremas y corto tiempo). Para evaluar la calidad y los parámetros de estabilidad oxidativa del aceite de canola que contiene MAG, se determinaron los ácidos grasos libres (AGL), el índice de peróxido (IP) y el índice de estabilidad oxidativa (OSI). Los resultados indicaron que con el aumento de los niveles de MAG, los AGL aumentaron de 0.05 hasta 0.2%. El IP mostró un aumento de 2 a 100 meq/L a medida que aumentaba la concentración de MAG. Además, se demostró que proporcionalmente a la concentración de MAG, el OSI aumentó de 12.20 a 13.10 h.

PALABRAS CLAVE: *Aceite de canola; Estabilidad oxidativa; Monoacilgliceroles; Prueba de Rancimat; Prueba del horno Schaal*

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1. INTRODUCTION

In addition to triacylglycerols (TAGs) as major constituents, edible oils contain minor components, which in spite of their lower contents, have a marked effect on physicochemical properties (Chen *et al*., 2011). Monoacylglycerols (MAGs), diacylglycerols (DAGs), free fatty acids (FFAs) and phospholipids are important minor components which are present in oils (Caponio *et al*., 2011, Aubourg, 2001, Paradiso *et al*., 2010, Mistry and Min, 1987). Such components, especially MAGs, DAGs, and FFAs exist inherently in oil; although exposing the oil to unfavorable conditions, such as high temperature, moisture and/or the enzymatic activity of lipases results in the generation of said compounds (O'Brien, 2008). MAGs are produced via the partial hydrolysis of TAGs. In fact, they are the monoester of a glycerol molecule, esterified by only one fatty acid. Due to the presence of two free hydroxyl groups on the MAG structure, it can modify both the physicochemical and oxidative stability of oils (Coleman and Lee, 2004). It is worth noting that, depending on their fatty acid composition, which may be rather saturated and/or unsaturated, the effects of MAGs may vary. (Naderi *et al*., 2016). One of the most important impacts of MAGs is on the oils' oxidative stability (Gomes *et al*., 2010, Caponio *et al*., 2011). Such impacts are connected to the fatty acid composition of oils and presumably that of MAGs (Paradiso *et al*., 2014). The oxidation reaction notably affects the quality and safety of oils (Jalili *et al.,* 2018). The products generated in the oxidized oil are hydroperoxides (primary oxidation products), ketones, aldehydes and epoxides (secondary oxidation) and also many free radical species (Taghvaei and Jafari, 2015). Furthermore, lipid oxidation leads to the production of toxic compounds. A study on the oxidized oils distinguished their negative impacts on human health. In this context, the development of liver tumors and the formation of 8-hydroxy-deoxyguanosine in the liver DNA of mice were observed by feeding them oxidized lard, soybean and sardine oils (Ichinose *et al*., 2004). On the other hand, high dosage of oxidized cod liver oil and lard, besides impaired fertility in female rats, caused morphologically abnormal spermatozoa in male rats (Zidkova *et al*., 2004).

As mentioned above, MAGs affect the oxidative stability of oils. However, inconsistent results were reported by different research groups in this context. It was shown by Gomes *et al*., (2010) that unsaturated MAGs (1–3 wt %) increased the induction period (IP) of oxidation in olive oil. According to Mistry and Min (1988), MAGs showed pro-oxidative effects on soybean oil which was attributed to the increasing effect of MAG on oxygen solubility. The pro-oxidative effects of MAG on soybean oil were also observed by Colakoglu (2007). Chen *et al*., (2014) studied the influence of MAGs and DAGs on the antioxidant activity and oxidative stability of stripped soybean oil. They concluded that MAGs (0–2.5 wt %) had no remarkable effect on the chemical stability of this oil. It was shown by Caponio *et al*., (2011) that the effect of MAGs on the oxidative stability of soybean oil was dose-dependent. Paradiso *et al*., (2014) investigated some behaviors of unsaturated MAGs from soybean oil in different oils. In their research, to study the role of fatty acid composition, MAGs were added into purified palm, olive, peanut, sunflower and soybean oils at levels of 0.5, 1.0, 2.0 and 3%, and then submitted to heat in the oven at 60 °C for 18 days. The findings of this research indicated that the antioxidant activity of MAG was variable in the oils with different fatty acid compositions.

All the above-mentioned works involve the use of nearly unsaturated MAGs. Considering that the saturated MAGs are extensively used as an emulsifier as well as structuring agent in the manufacture of food products, there is a lack of data on the impact of saturated MAGs on the oxidative stability of oils. In this paper, we discuss the effects of saturated MAGs on the oxidative stability of canola oil at a moderate temperature and over a long time (MTLT, Schaal oven test at 60 °C) and a high temperature and short time (HTST, Rancimat test at 110 °C).

2. MATERIALS AND METHODS

2.1. Materials

Refined, bleached and deodorized canola oil was purchased from a local market in Tehran (Iran) and stored at refrigerator temperature (5 °C) until used. MAG (composed of 90–95% 1-MAG, 3–5% 2-MAG, 2–4% DAG, less than 1% glycerol, and free fatty acids) with the commercial name of BLANID DMAG 1600 was purchased from Farzan Rad Co. (Tehran, Iran). Other chemicals were obtained from Merck Co. (Darmstadt, Germany).

2.2. Sample preparation

MAG (at 0, 0.5, 3 and 5 % levels) was dissolved in canola oil with slow agitation (50 rpm) and the resulting oil was stored in a refrigerator until analysis.

2.3. Fatty acid profile analysis

Fatty acid methyl esters were prepared according to the American Oil Chemists' Society (AOCS) Ce 2-66 method and analyzed by a Trace GC (Yanglin, South Korea) according to AOCS Ce 1-91 method (AOCS, 1996). The injection was performed in a split ratio of 1:80. The carrier gas was nitrogen at a flow rate of 0.8 ml/min. A BPX-70 capillary column (60 m × 0.25 mm × 0.25 mm, Restek, Bellefonte, PA, USA) and a flame ionization detector were used to resolve and detect fatty acid methyl esters. The oven temperature was set at 175 °C for 30 min, then programmed to increase to 200 °C at a rate of 1.5 °C/min.

2.4. Determinations of oxidation indexes in MTLT and HTST conditions

2.4.1. MTLT (Schaal oven test)

The Schaal oven test was conducted by the incubation of samples at 60±1 °C for 31 days (Gomes *et al.*, 2010). Ther peroxide value (PV) and free fatty acid (FFA) contents of samples were measured in 3-day intervals. PV was determined according to the AOCS method Cd 8-53 (AOCS, 1996). FFA was determined as a percentage of oleic acid in accordance with AOCS Official Method Ca 5a-40 (AOCS, 1996). The induction period of oxidation (IPOx) and oxidation rate ($Rate_{ox}$) of samples at 60 °C were calculated using PV data. For this purpose, the data were fitted with the modified Gompertz equation (Eq. 1) and IPOx and $Rate^{ox}$ were calculated using equations 2 and 3, respectively (Farmani and Gholitabar, 2015).

$$y = A + Ce^{-e^{-B(t-M)}} \quad \text{(Eq. 1)}$$

A is the asymptotic Y (oxidation content) as t (time) decreases indefinitely, C is the asymptotic Y that occurs as t increases indefinitely, and B is the relative oxidation rate at M, where M is the time at which the absolute oxidation rate is maximum.

Parameters B, A, C, and M were calculated using the Sigma plot ver.12 (Systat Software Inc.,) for each PV curve, and an IPOx and $Rate_{ox}$ were calculated from the following equations:

$$IP_{ox}=M-1/B \quad \text{(Eq. 2)}$$

$$Rate_{ox}= (B\times C)/e \quad \text{(Eq. 3)}$$

2.4.2. HTST (Rancimat test)

A Metrohm Rancimat instrument, model 743 (Herisau, Switzerland) was used to determine the oil stability index (OSI) at 110 °C according to the AOCS method Cd 12b-92 (AOCS, 1996). Sample size and air flow rate were 2.5 g and 2.5 ml/s, respectively.

2.5. Statistical analysis

One-way ANOVA was used to analyze the data statistically using SPSS version 16.0 (SPSS Inc. Chicago, IL, USA). The Duncan test was used to understand the significant differences between means at a $p < 0.05$ level. All data were shown as mean value ± standard deviation of triplicate experiments.

3. RESULTS AND DISCUSSION

3.1. Fatty acids profile

The fatty acid composition of canola oil as affected by saturated MAGs is presented in Table 1. As presented in this Table, the predominant fatty acids in canola oil were oleic acid (60.5%) and linoleic acid (19.5%). The MAG used in this work was almost fully saturated (99.5 % SFA) and contained palmitic acid (96.5 %) as the main fatty acid (Table 1). The fatty acid composition and production method of the MAG used in this work were compared with those of other papers and presented in Table 2. As can be seen in the Table, the MAG used in this study was almost fully saturated whereas those used in other works were mainly unsaturated. MAG addition in a concentration of 0.5% did not affect the fatty acid composition of canola oil; however, the addition of MAGs at levels of 3.0 or 5.0% to canola increased the content of palmitic acid (C16:0) ($p < 0.05$), whereas the percentages of stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2) decreased significantly ($p < 0.05$) from 3.7, 60.5 and 19.5% to 3.5, 57.4 and 18.5%, respectively.

The study of Paradiso *et al.*, (2014) revealed that the fatty acid composition of MAG plays an important role in its effect on the oxidative stability of oils. It was shown that the antioxidant effect of MAGs was greater in oils with a lower unsaturation degree (palm and olive oil) rather than in those having higher amounts of unsaturated fatty acids (peanut, sunflower and soybean oil). Our recent research revealed that the addition of 3.0 or 5.0% saturated MAGs to sunflower oil and chicken fat (with SFA of 30% and palmitic acid of 23.0%) increased its PV and FFA (Naderi *et al.*, 2015; Naderi *et al.,* 2016). Though the fatty acid compositions of chicken fat and sunflower oil were different, their PV or FFA curves (in a Schaal oven test at 60 °C) presented the same trend as affected by saturated MAGs (Naderi *et al*., 2016; Naderi *et al*., 2015). These findings indicate that the impact of saturated MAGs is not associated with fatty acid composition.

3.2. Free fatty acids

Percent FFA is an important quality indicator. The smoke point of oil decreases with the increase in percent FFA (Frega *et al.,* 1999). Deodorized oils generally have an FFA level of less than 0.05% (O'Brien, 2008). As shown in Figure 1, the FFA curves of the sample containing 0.5, 3.0 and 5.0 MAG as well as canola oil showed the same trend up to the 21st day, but the canola oil with the 3.0 and 5.0% MAG showed an incremental trend

Table 1. Effect of monoacylglycerol (MAG) addition on fatty acid composition and iodine value (IV) of canola oil

Samples	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	SFAs
Canola oil	0.30±0.1[a]	6.9±0.1[b]	3.7±0.2[a]	60.5±0.1[a]	19.5±0.1[a]	6.7±0.1[a]	10.6±0.1[c]
CO 0.5 %	0.27±0.2[a]	7.2±0.1[b]	3.7±0.1[a]	61.0±0.1[a]	19.4±0.1[a]	6.6±0.1[a]	11.0±0.1[c]
CO 3.0 %	0.21±0.2[a]	9.4±0.1[a]	3.6±0.2[a]	58.6±0.1[b]	18.9±0.2[b]	6.4±0.2[a]	13.2±0.1[b]
CO 5.0 %	0.14±0.1[a]	11.3±0.1[a]	3.5±0.2[a]	57.4±0.1[b]	18.5±0.2[b]	6.3±0.2[a]	15.0±0.1[a]
MAGs	2.5±0.1	96.5±0.1	0.3±0.1	0.3±0.1	0.0	0.0	99.5±0.1

Data are presented as the means ± SD of three replicates according to the Duncan test. Different superscripts show significant differences in each column at $p < 0.05$. CO: Canola Oil. SFAs: Saturated Fatty Acids. MAGs: Monoacylglycerols

Table 2. The MAGs applied in some papers mentioned in this study

	References			
Fatty acids (%)	Caponio *et al.*	Paradiso *et al.*	Gomes *et al.*	This work
C14:0	0.51	0.29±0.04	0.02	2.5±0.1
C16:0	11.33	14.29±0.27	9.0	96.5±0.1
C18:0	3.29	3.02±0.43	2.61	0.3±0.1
C18:1	27.87	29.47±0.69	77.9	0.3±0.1
C18:2	39.37	47.03±1.02	8.5	0.0
C18:3	4.21	4.18±0.23	0.57	0.0
SFAs	16.11	17.97	12.13	99.5
MAG Production Method	Saponification	Saponification	Saponification	Distillation

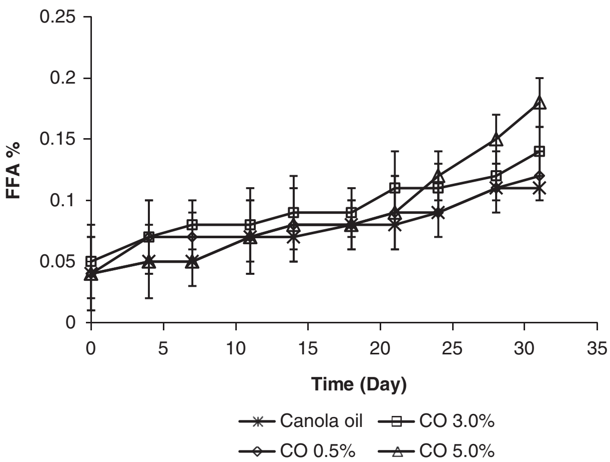

Figure 1. The free fatty acid (FFA) of canola oil samples containing 0.05, 3.0 and 5.0% monoacylglycerols (MAGs) determined for 31 days at 60 °C; CO: canola oil. Results are expressed as mean ± SD of three replicates.

thereafter. The increase in percent FFA in MAG (3–5%)-containing canola oil could be attributed to the hydrolysis of the oil at the test condition. In fact, as reported by Zhang *et al.,* (2015) and Krishnamurthy (1982), partial acylglycerol compounds like MAG or DAG had a catalytic effect on the hydrolysis TAGs, resulting in a FFA increase. Vichi *et al.,* (2003) also reported that a gentle heat condition and prolonged time could raise hydrolytic rancidity in the oils. A similar result on the effect of MAG on the percent FFA of chicken fat and sunflower oil was previously reported by Naderi *et al.*, (2015) and Naderi *et al.*, (2016). However, the findings of Gomes *et al.*, (2010) were not in accordance with our study. They reported that MAG addition to purified olive oil caused the FFA to decrease. This opposite results can be attributed to the difference in composition, the purity and the production method of MAG (Table 2). It is necessary to mention that the MAGs used in our study were obtained by distillation (from palm oil) but those used in the study of Gomes *et al.*, (2010) were obtained through saponification (from purified olive oil).

The increase in FFA content may accelerate the oxidation of the oil. Mistry and Min (1987) reported that the addition of FFA into soybean oil products pro-oxidative effects on the oil. Accordingly, they concluded that the free carboxyl groups of FFA and the hydroxyl groups of MAG were responsible for such phenomena, such as octadecane, which lacks a free carboxylic group and did not show any pro-oxidative effects. In general, the increase in MAGs and DAGs in oils during storage is an indicator of the hydrolysis occurring. After hydrolysis, esterified fatty acids become free. Miyashita and Takagi (1986) reported that FFAs that have a free hydroxyl group show a pro-oxidative effect but the methylated ones had no impact on oxidative stability. Hydrolysis needs a long exposure time to occur, providing enough energy to start the oxidation is available (Zhang *et al.*, 2015). In this paper, the Schaal oven test was applied, nearly simulating the required conditions (MTLT) for hydrolysis to take place. Paradiso *et al.*, (2010) pointed out that the addition of higher levels of FFA (2.0 and 3.0%) to purified olive oil caused a decrease in the oxidized TAG content; whereas the addition of lower levels of FFA (0.5 or 1.0%) caused an increase in the oxidized TAG content. They attributed this result to the decomposition of hydroperoxides. In fact, by increasing the content in FFA, hydroperoxides will be broken-down more, therefore decreasing the PV.

3.3. Oxidative stability

To determine the oxidative stability of canola oil as affected by saturated MAGs, PV (for 31 days) and the Rancimat test were evaluated as representative of MTLT and HTST conditions, respectively.

3.3.1. Peroxide value

The PV curves of canola oil samples obtained by the Schaal oven test (60 °C) are illustrated in Figure 2. No significant differences were found among samples till the 15th day; after that, however, the PV of a sample containing 3.0 and 5.0% MAG was significantly higher than the others. Ultimatelly, with the increase in time and MAG concentration in the samples, PV increased more rapidly. The same results were obtained in our previous research on sunflower oil and chicken fat as affected by 0.5, 3.0, and 5.0% saturated MAGs (Naderi *et al.*, 2015, Naderi *et al.*, 2016).

To investigate the oxidation kinetics (IP_{ox} and $Rate_{ox}$) of the samples quantitatively, and also better comparing the PV data (obtained at 60 °C, MTLT) and OSI (obtained at 110 °C, ETST), the PV data were fitted with the modified Gompertz equation and IP_{ox} and $Rate_{ox}$ were calculated (Table 3). As shown in Table 3, no significant differences were found between the sample containing 0.5% MAGs and the control in terms of their IP_{ox} or $Rate_{ox}$ ($p > 0.05$). However, at a 5.0% MAG level, shorter IP_{ox} and higher $Rate_{ox}$ were observed ($p < 0.05$). Chen *et al.*, (2014) stated that MAG addition to the soybean oil increased the antioxidative efficiency of alpha-tocopherols. To prove such a phenomenon, they added 40 μM alpha tocopherols to soybean oil containing 0.5% MAG and compared the oxidation lag phase (at 55 °C) of this system with a blank (without MAG). It was distinguished that the lag phase of both systems was the same (1 day). However, soybean oil containing 2.5% DAG and 40 μM alpha tocopherols had a longer lag phase (4 days) than the blank and soybean oil with 0.5% MAG. The research of Caponio *et al.*, (2011), carried out on purified soybean oil, revealed that MAGs showed pro-oxidative effects at lower concentrations, and antioxidative effects at higher levels.

TABLE 3. Oxidative stability index (OSI) at 110 (°C) and oxidation kinetics of canola oil (CO) samples determined by the Schaal oven test at 60 °C

		Oxidation Kinetics at 60 °C	
Samples	**OSI at 110 (°C)**	**$IP_{OX\ (day)}$**	**$Rate_{ox\ (1/min)}$**
Canola oil	12.20±0.4[a]	26.2±0.06[a]	0.90±0.06[b]
CO 0. 5%	12.30±0.3[a]	26.0±0.06[a]	0.92±0.05[b]
CO 3.0%	12.70±0.7[a]	15.2±0.06[b]	13.1±0.06[a]
CO 5.0%	13.10±0.6[a]	14.7±0.06[b]	27.2±0.04[a]

Data are presented as the means ± SD of three replicates according to Duncan test. Different superscripts show significant differences in each column at $p < 0.05$. IP_{ox}: Induction period of oxidation.

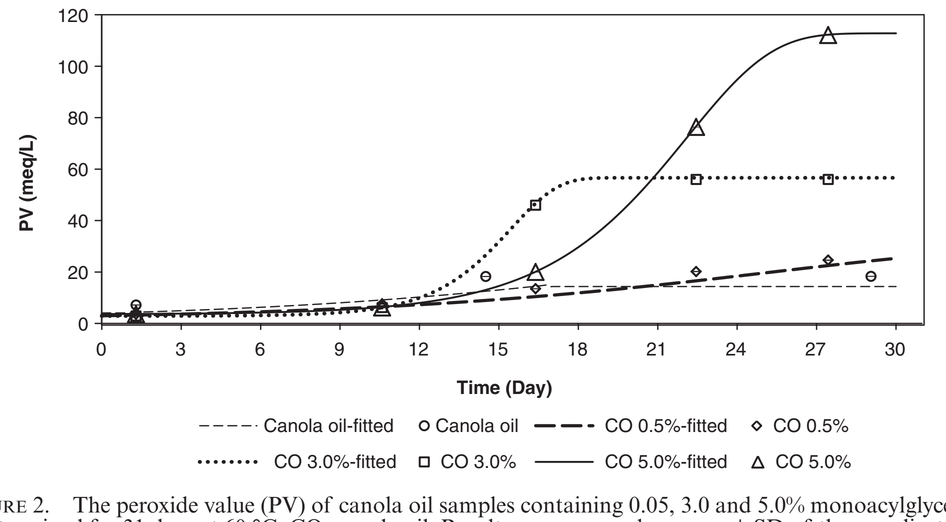

FIGURE 2. The peroxide value (PV) of canola oil samples containing 0.05, 3.0 and 5.0% monoacylglycerols determined for 31 days at 60 °C; CO: canola oil. Results are expressed as mean ± SD of three replicates.

These findings are connected to the fact that MAGs at lower concentrations could decrease the surface tension of oils and therefore increase oxygen diffusion. On the other hand, at higher levels, the layer of MAGs present on the surface interact with most of the inlet oxygen, thus not allowing further oxygen to reach the TAGs, resulting in the deceleration of the oxidation rate. Contrary to the results of Caponio *et al*., (2011), as observed in our study, MAGs at higher concentrations accelerated the rate of oxidation. This inconsistency could be explained by the difference in the fatty acid compositions of the MAGs used in the two studies (Table 2). In fact, due to the almost fully saturated nature of the MAG applied in the current paper, it interacts with little oxygen (due to the lack of double bonds in the fatty acid structure of the MAGs) and therefore a high amount of oxygen can interact with TAGs (Paradiso *et al*., 2014). The reports of Colakoglu (2007) and Mistry and Min (1988) showed that the MAG at a lower concentration (0.5%) had a pro-oxidant effect and could accelerate fat oxidation by the interaction of oxygen with TAGs present in oils throug their free hydroxyl groups. Surprisingly, in contrast to the findings of Caponio *et al*., (2011), Gomes *et al*., (2010) reported that as the concentration of MAGs increased, the PV of purified olive oil decreased. Due to the similarity of the SFA content of the MAGs used in both papers, this peculiar result could not be explained by the fatty acid composition of MAGs. It might be due to some antioxidant compounds in olive oil, entering into the extracted MAGs during the preparation (by saponification) process of MAGs by Gomes *et al*., (2010).

3.3.2. Rancimat test

To identify the impacts of MAGs under the HTST condition, the Rancimat test, which is the automated method of the active oxygen method, was applied (Velasco and Dobarganes, 2002). As illustrated in Table 3, there is no significant difference between canola oil and the MAG-added samples ($p > 0.05$). However, as discussed in the previous section, at a MTLT condition, MAG addition caused a decrease in the oxidative stability of canola oil. The inconsistency of the Schaal oven test (MTLT) and Rancimat (HTST) results could be interpreted by different oxidation mechanisms at moderate and high thermal conditions. Considering that the Rancimat test is a fast method and the oils are not exposed to heat and oxygen for a prolonged time, glycerolysis does not occur (Zhang *et al*., 2015). As mentioned for FFA, by long exposure of oil to MTLT conditions (Schall oven test), glycerolysis happens, which subsequently provides the required energy for oxidation. On the other hand, the increase in OSI by MAG addition could be explained by the fatty acid composition (as discussed previously). It is necessary to mentioned that during the Rancimat test, formic acid is produced as a result of the breakdown of the secondary oxidation products (like aldehyde and ketone) and increases the electrical conductivity of the measuring vessel. In general, higher contents of saturated fatty acids could postpone the generation of formic acid, leading to the increase in OSI (O'Brien, 2008).

Unlike our current results, Gomes *et al*., (2010) reported that the oxidative stability of olive oil (at 85 °C) was increased by MAG addition (1, 2 and 3 wt %). Since the fatty acid composition of MAGs used in the Gomes *et al*., (2010) study consisted of mainly unsaturated fatty acids (Table 2), the increase in the oxidative stability of olive oil cannot be attributed to the increase in the SFA content of the MAG-containing oil. As mentioned before, the improvement of quality and oxidative stability indexes (FFA, PV and OSI) by MAG addition, reported by Gomes *et al*., (2010), are attributable to the antioxidant compounds which may enter into the oil along with the MAGs (which were prepared by the saponification of olive oil).

4. CONCLUSIONS

Overall, the current paper shows some contradictions to previous research conducted in this content. These contradictions are largely connected to the fatty acid composition of the MAGs and oils, the production methods for obtaining MAGs, and also the time/thermal conditions the oils were exposed to, such as MTLT (Schaal oven test at 60 °C for 31days) or ETST (Rancimat test at 110 °C for 1 day maximum). The finding in our study indicate that the oxidative stability and quality indexes (PV and FFA) at 60 °C were attenuated as MAG concentration increased; however, when exposing to ETST (Rancimat test), the OSI of samples showed an increasing trend. The Opposing results of the Schaal oven test and Rancimat test are attributable to the different mechanisms which occurred under moderate and extreme heat conditions. It was determined that MAGs produce a pro-oxidant effect as a result of the initial glycerolysis of oils at the temperature ranging from 50–60 °C and prolonged time. On the other hand, the major reason for the inconsistency between our study and others is the difference in the SFA of the applied MAGs. As mentioned before, other works have used nearly unsaturated MAGs whereas our applied MAGs were saturated. Investigation into the oxidation process of other oils as affected by saturated MAGs requires further study.

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