Short Note

The monoacylglycerol fatty acid composition can act in modulating the oxidative process

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This work was aimed at evaluating the effect of the addition of monoacylglycerols (MAG) with different fatty acid composition, thus obtained from different vegetable oils such as olive (OO), soybean (SO), and palm (PO). MAG were added in the purified olive oil at different concentrations (5, 15, and 30 mg/g) and consequently the oxidative process was monitored. For this purpose, the purified oil added with MAG was subjected to oven test for 28 days, during which the multiparametric analysis of oxidation was carried out: peroxide value, spectrophotometric constants (K_{270}, K_{232}), oxidized triacylglycerol, and triacylglycerol oligopolymers. Our results showed that both OO and SO MAG significantly slowed the oxidative processes after 15 days, in a dose-dependent manner. For PO MAG, on the other hand, although the dose dependent effect was less evident, an antioxidant effect was observed that was greater than that observed for the other MAG, already detectable in the early stages of oven test.

Keywords: monoacylglycerols, oxidative stability, oven test, vegetable oils

1. INTRODUCTION

Lipid oxidation is a free radical chain reaction between unsaturated fats and oxygen that can occur in an autocatalytic manner. The overall mechanism of lipid oxidation involves three stages: initiation, propagation, and termination. In the initiation step, a fatty acid radical known as the alkyl radical is formed by abstraction of a hydrogen from a fatty acid in the presence of an initiator. In bulk oils, the formation of alkyl radicals in fatty acids increases with increasing unsaturation. The greater the degree of unsaturation of fatty acids, the greater the susceptibility to the initiation phase [1-4]. The propagation phase consists of a series of oxidation reactions that lead to free radicals ($R^{\bullet}$ and $ROO^{\bullet}$) and hydroperoxides (ROOH). The final products are aldehydes and ketones, some of which are responsible for the rancid flavour, and triacylglycerols oligopolymers (TAGP). The oxidation rate of lipids can induce the reduction of the nutritional value and the formation of potentially toxic molecules [5].
Monoacylglycerols (MAG) are monoesters of glycerol in which one of the hydroxyl groups is esterified with a fatty acid. These molecules are characterised by hydrophobic and hydrophilic domains that give them emulsifying properties. Emulsifiers are able to determine the properties of the emulsion playing a key role in the acceptability level of foods as they can bind aromatic compounds [6]. MAG are the most commonly used emulsifiers in the food industry (creams, spreads and ice cream) [7].
Several studies focused on the effect of MAG on vegetable oils oxidative stability. Mistry & Min [8] showed a pro-oxidant effect of SN-α-monolinolein on purified

soybean oil. The effect on the oxidative stability of soybean oil was determined by measuring peroxide values and the volatile compound formation by gas chromatography. The same authors [9] measured the disappearance of headspace oxygen in purified soybean oil containing 0, 0.25 and 0.5% monostearin, distearin, monolinoelin or dilinolein during storage at 55°C and attributed the pro-oxidant effect to ability of the hydrophilic groups to arrange on the surface of the oil and accelerate the solubility of oxygen, thus increasing the oxidation rate. Further, Colakoglu [10] evaluated the oxidation kinetics of soybean oil added with monoolein, stearic acid, and iron concluding that MAG had a pro-oxidant effect. Instead, Gomes et al. [11] showed a marked antioxidant effect of MAG in purified olive oil, contrary to what has been observed by other authors, explaining that this result could depend on the different fatty acid composition of the oil in which MAG were added. Moreover, Caponio et al. [12] showed that the MAG addition in purified soybean oil exert a dose-dependent effect. In fact, the addition of low amounts of MAG (5-10 g/kg) caused an increase of oxidation, whereas adding higher amounts of MAG, the pro-oxidant effect progressively decreased. Thus, MAG acted as a pro-oxidant when added in low quantities (up to 1%) – confirming the data reported in literature [8-10] – and as an antioxidant when added in larger quantities. Paradiso et al. [13] obtained that the antioxidant effect exerted by MAG was significantly higher in less unsaturated oils, such as palm and olive oils. Moreover, among the more unsaturated vegetable oils, peanut and sunflower oils showed an almost linear slowdown of oxidation, slightly less pronounced in sunflower oil, which was the most susceptible to oxidation due to its high content of linoleic acid. A peculiar trend was highlighted for soybean oil, where the antioxidant effect of high amounts of monoacylglycerols was opposed to a pro-oxidant effect observed up to 1%.
Based on these considerations, our work was aimed at evaluating the effect of the MAG fatty acid composition on the oxidative stability of a purified olive oil. In fact, the comprehension of the mechanism of action of MAG in oxidative phenomena deserves attention since they are used in foods as emulsifiers.

2. MATERIALS AND METHODS

2.1 PREPARATION OF PURIFIED OILS

Olive, soybean, and palm oils purchased from local retail were used for the preparation of the respective purified oils using the method described by Lee and Min [14] with some slight modifications as reported in Paradiso et al. [15]. The obtained purified oils were, then, free of MAG, free fatty acids, tocopherols, phospholipids, and oxidized compounds [16].

Table I – Fatty acid composition and chemical analyses on purified olive oil.

Determinations	Value
Fatty acid composition (%)	
$C_{12:0}$	0.00
$C_{14:0}$	0.00
$C_{16:0}$	10.46
$C_{16:1}$	0.32
$C_{17:0}$	0.06
$C_{18:0}$	2.24
$C_{18:1}$	79.25
$C_{18:2}$	6.29
$C_{18:3}$	0.44
$C_{20:0}$	0.34
$C_{20:1}$	0.48
Others	0.14
Chemical analyses	
FFA (g/100 g)	0.00
PV (meq O_2/kg)	0.00
K_{232}	0.851
K_{270}	0.022
TAGP (g/100 g)	0.009
ox-TAG (g/100 g)	0.009
DAG (g/100 g)	0.000
PC (g/100 g)	0.018

FFA: free fatty acids; PV: peroxide value; K_{232}: absorbance at 232 nm; K_{270}: absorbance at 270 nm; TAGP: triacylglyceol oligopolymers; ox-TAG: oxidized triacylglycerols; DAG: diacylglycerols; PC: polar compounds.

Table II – Fatty acid composition of MAG from olive oil (OO), soybean oil (SO), and palm oil (PO).

Fatty acids (%)	OO	SO	PO
$C_{12:0}$	0.00	0.00	0.06
$C_{14:0}$	0.00	0.08	0.70
$C_{16:0}$	8.49	10.77	35.15
$C_{16:1}$	0.04	0.07	0.22
$C_{17:0}$	0.24	0.11	0.05
$C_{18:0}$	2.64	5.99	4.42
$C_{18:1}$	80.82	30.49	46.74
$C_{18:2}$	5.93	47.09	11.34
$C_{18:3}$	0.33	4.20	0.10
$C_{20:0}$	0.49	0.54	0.44
$C_{20:1}$	0.64	0.03	0.24
Others	0.39	0.66	0.54
Iodine value*	85	115	64

* Calculated as reported by Kyriakidis and Katsiloulis [19].

2.2 PREPARATION OF MAG

Purified MAG were obtained by the partial saponification of an aliquot of respective purified oil as reported in a previous work [13]. MAG were obtained with a purity percentage of 93.5%, 99.5%, and 96.1% for olive (OO), soybean (SO), and palm (PO), respectively, as confirmed by the High-Performance Size-Exclusion Chromatography (HPSEC) analysis. Purified olive oil was used as a control (C). The MAG obtained were added to the purified olive oil in the following proportion MAG/C: 0.5% (MAG-5 mg/g); 1.5% (MAG-15 mg/g); 3% (MAG-30 mg/g).

2.3 OVEN TEST

Three grams of C were weighed in an 8 mL glass vials and subjected to a constant temperature of 60°C in a ventilated oven for 28 days. Eight vials for each time (two for C and two for each MAG/C mixture) were tested.

2.4 CHEMICAL ANALYSES

The determinations of the free fatty acids (FFA), peroxide value (PV), and spectrophotometric constants were carried out according to the Official Journal of the European Communities [17]. Fatty acids composition was carried out by gas chromatographic analysis of fatty acid methyl esters as reported in a previous work [18].

The iodine value (IV) was determined as proposed by Kyriakidis and Katsiloulis [19] that uses the percentage of fatty acid methyl esters determined from the analysis of the fatty acid composition and an equation with coefficients specific for every type of vegetable oil. The proposed equation is:

$$IV = xC_1 + yC_2 + zC_3$$

where C_1, C_2, and C_3 correspond to the sum of the relative percentage concentrations of the unsaturated fatty acids with one, two, and three double bonds respectively, whereas x, y, and z are the relative coefficients.

TAGP and ox-TAG were determined by means of HPSEC of polar compounds (PC), previously separated from the oil samples by silica gel column chromatography according to AOAC method [20].

The identification and quantification of individual peaks was carried out as described in a previous paper [21]. Three replicates were analysed per sample for each determination.

2.5 STATISTICAL ANALYSIS

An analysis of variance (three-way ANOVA) was carried out on the experimental data by using XLStat software (Addinsoft SARL, New York, NY, USA). The ANOVA was performed considering the amount of MAG added to the purified olive oil (MAG%), the different vegetable oils considered (MAG-oil) and the time, as well as their interaction, as independent variables. Tukey's HSD test was applied for multiple comparisons.

3. RESULTS AND DISCUSSION

3.1 CHARACTERISATION OF OIL AND MAG

Table I shows the fatty acids composition of purified olive oil used in oven test and the results of the chemical analyses carried out to verify the purity level. The fatty acid composition reported corresponded to the typical composition of olive oils [22], while chemical analyses confirmed that purified oils were almost completely composed of unaltered triacylglycerols (>99,8%). Moreover, free fatty acids and peroxide values were close to zero, and the spectrophotometric constants showed low values, similar to those detected in our previous paper [16].

Table II shows the fatty acid composition of the different MAG used in experimental tests, which are in accordance with their botanical origin [22]. In detail, olive oil MAG showed a higher monounsaturated fatty acids content, instead soybean oil's MAG were richer in polyunsaturated fatty acids and, lastly, palm oil's MAG exhibited a higher saturated fatty acids content than the other MAG. Difference in MAG acidic composition was overall summarised in iodine value, higher in soybean oil than olive and palm.

3.2 OVEN TEST

Table III shows the results of the analysis of variance (three-way ANOVA) performed on analytical parameters data detected. ANOVA model was significantly different ($p < 0.001$) for all the parameters. Time and MAG-oil variables had highly significant effect on all the indices ($p < 0.001$), while the variable MAG% had a significant effect on all the indices except K_{270}. This trend was also observed in the second-level interaction *Time*MAG%*MAG-oil*. The first-level interaction *Time*MAG%*, *Time*MAG-oil*, and *MAG%*MAG-oil* were significant for all the parameters detected.

Figure 1 reports average values of peroxide content obtained by analysis conducted on C and the same added with MAG-5, MAG-15 e MAG-30 of olive, soybean and palm oils during oven test at 60°C. After 15 days it was observed that an increasing concentration of OO and SO MAG oxidation process slowed down oxidative processes in a significant manner ($p < 0.05$). In the samples added with PO MAG, characterised by a major content of saturated fatty acid and consequently a lower iodine value, the highest inhibiting effect was observed. However, the dose-dependent effect of MAG was less evident. The lowest protective effect on triacylglycerol oxidation was exerted by OO MAG. In fact, OO MAG had a fatty acid composition like the purified olive oil used for trials. For this last case, after 24 days of oven test, the addition of MAG-5 mg/g showed pro-oxidative effect.

Naderi et al. [23] reported that the 5% MAG (of saturated fatty acids) addition in rapeseed oil led to acidity and peroxide value increase proportional to

Tabella III – Results of test of variance (three-way ANOVA and first order interaction) performed on analytical data

Parameters	Model		Time		MAG%		MAG-oil		Time*MAG%		Time*MAG-oil		MAG%*MAG-oil		Time*MAG%*MAG-oil	
	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
PV	532.852	< 0.001	3140.478	< 0.001	6.982	0.013	2283.383	< 0.001	76.653	< 0.001	417.758	< 0.001	81.410	< 0.001	22.840	< 0.001
K_{232}	111.172	< 0.001	647.020	< 0.001	6.138	0.018	514.854	< 0.001	13.975	< 0.001	88.772	< 0.001	14.456	< 0.001	3.937	< 0.001
K_{270}	10.229	< 0.001	60.144	< 0.001	1.362	0.300	14.931	< 0.001	5.204	< 0.001	9.843	< 0.001	5.840	< 0.001	1.040	0.433
TAGP	406.498	< 0.001	2087.492	< 0.001	5.005	0.031	1479.758	< 0.001	114.945	< 0.001	362.289	< 0.001	132.585	< 0.001	42.099	< 0.001
ox-TAG	483.410	< 0.001	2735.969	< 0.001	6.345	0.017	2101.311	< 0.001	82.615	< 0.001	388.069	< 0.001	88.210	< 0.001	24.452	< 0.001

PV, peroxide value; K_{232}, absorbance at 232 nm; K_{270}, absorbance at 270 nm; TAGP, triacylglycerol oligopolymers; ox-TAG, oxidized triacylglycerols.

MAG percentage added in. On the other hand, Gomes et al. [11] and Paradiso et al. [13] showed a marked antioxidant effect of MAG in purified olive oil. The authors reported that antioxidant effect of MAG was higher in oil with a low unsaturated level. These opposite results can be linked to the difference in MAG used by them. MAG used by Naderi et al [23] were obtained by distillation (from palm oil) but those used in the study of Gomes et al. [11] e Paradiso et al. [13] were obtained through a partial saponification process (from purified olive oil). Moreover, other authors studied the impact of MAG on the physical and chemical properties of stripped SO [24]. They concluded that MAG suppressed the effectiveness of α-tocopherol in SO, since MAG are able to significantly decrease the interfacial tension and form crystals in SO. MAG are surface-active molecules, with the ability to form physical structures in oils, like micellar structures, in presence of small quantities of water (~300 ppm) [3, 25]. It was observed that micellar structures are involved in lipid oxidation, especially during the propagation phase: they had an antagonist or synergic effect with hydroperoxides, showing an anti- or pro-oxidant MAG influence [26]. This trend was confirmed both by spectrophotometric constants and, above all, by ox-TAG and TAGP, that are specific indices of triacylglycerol oxidation (Figures 2 and 3). Despite the less marked differences observed for K_{270} value, the protective effect of MAG on triacylglycerol oxidation was statistically significant after 21 days. Once more this effect was much more evident in purified olive oil added with PO MAG. This result could be explained by the high saturation level of fatty acids, that could play a barrier effect on the oil/air surface, reducing oxygen input and preserving oil from oxidation. This point was recently confirmed by Wang et al [27], assuming that the antioxidant effect depends on a physical barrier formed by MAG, which limits the reaction between unsaturated fatty acid and oxygen. Moreover, it has been previously reported that MAG, having hydrophilic and hydrophobic groups in the same molecules, concentrate at the surface of the oil [10]. The observed antioxidant activity at the highest concentration of MAG could be due to the oxidation of the MAG layer at the surface, thus preventing triacylglycerols oxidation under the surface layer [12].

4. CONCLUSION

Data reported in this paper, showed that after 15 days of oven test OO and SO MAG at the highest concentration slowed down significantly the oxidative process as shown by the evaluated parameters. In case of PO MAG, despite the dose-dependency effect was less evident, an increasing was observed in the antioxidant effect than the other MAG, already in the first stage of the oven test. Moreover, the OO MAG showed less

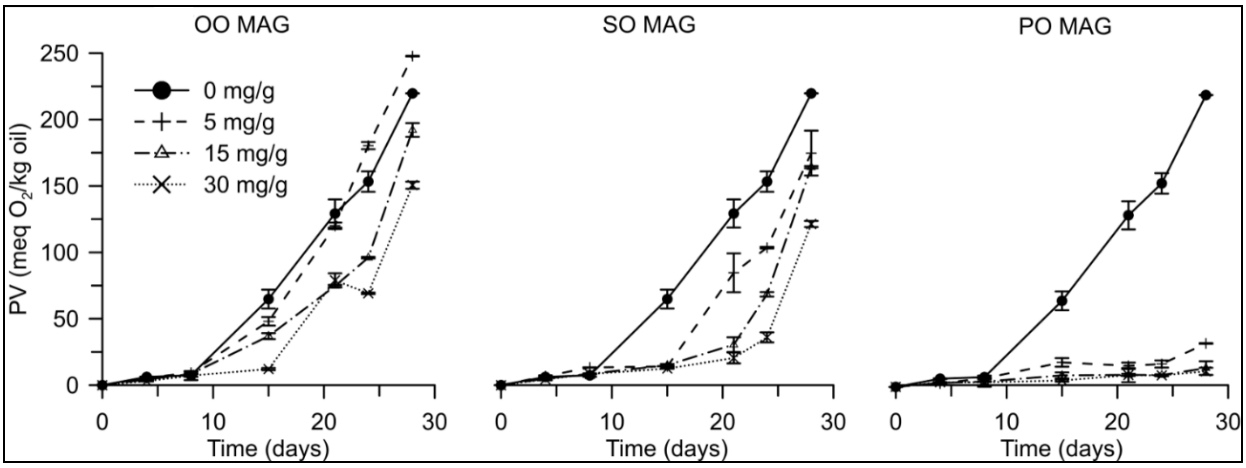

Figure 1 - Determination of peroxide value (PV) in purified olive oil and in the same added with 0.5%, 1.5%, and 3% of monoacylglycerols (MAG-5 mg/g, MAG-15 mg/g, MAG-30 mg/g) of olive oil (OO), soybean oil (SO), and palm oil (PO) during the oven test at 60°C.

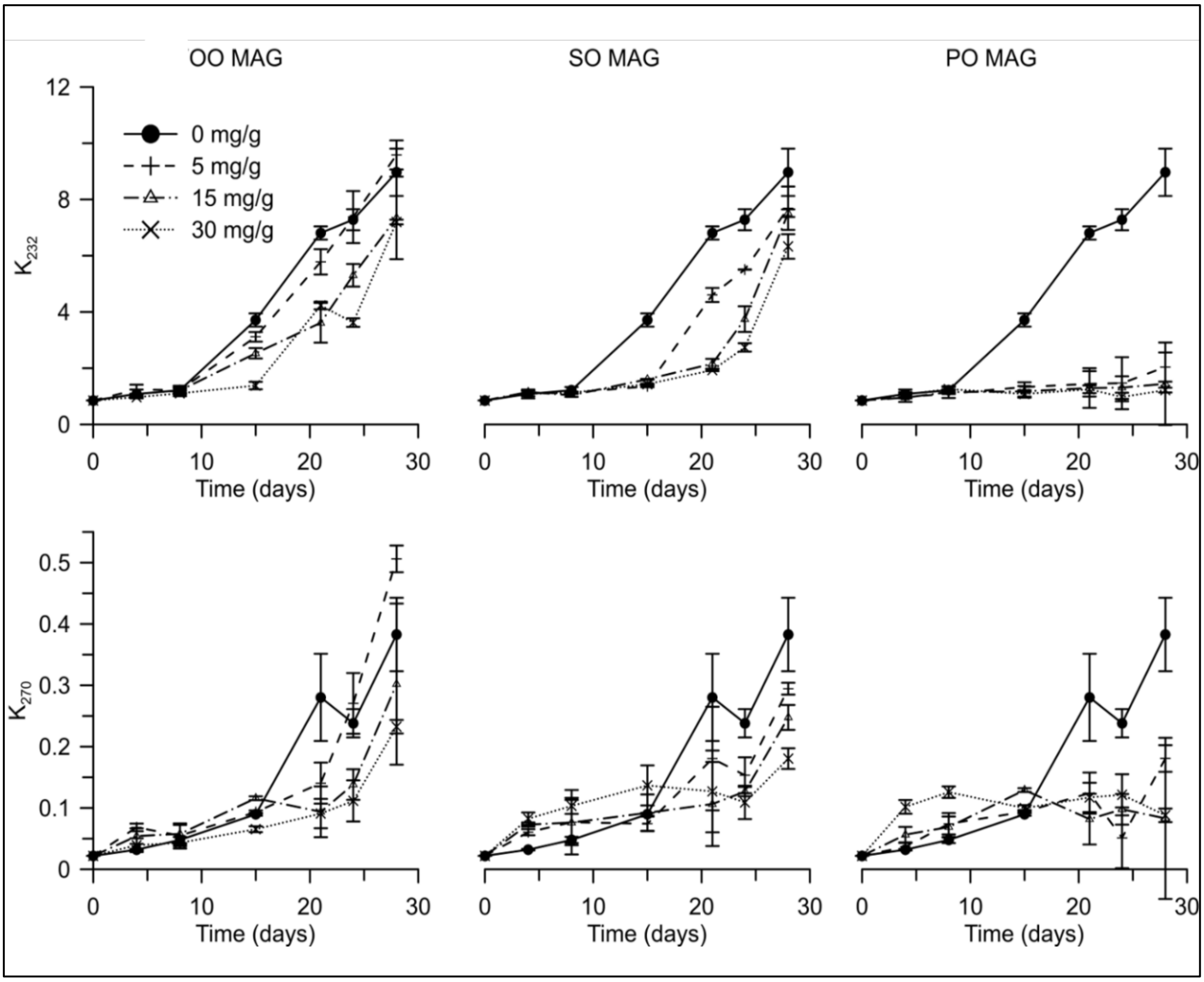

Figure 2 - Determination of spectrophotometric constants (K_{232}, K_{270}) in purified olive oil and in the same added with 0.5%, 1.5%, and 3% of monoacylglycerols (MAG-5 mg/g, MAG-15 mg/g, MAG-30 mg/g) of olive oil (OO), soybean oil (SO), and palm oil (PO) during the oven test at 60°C.

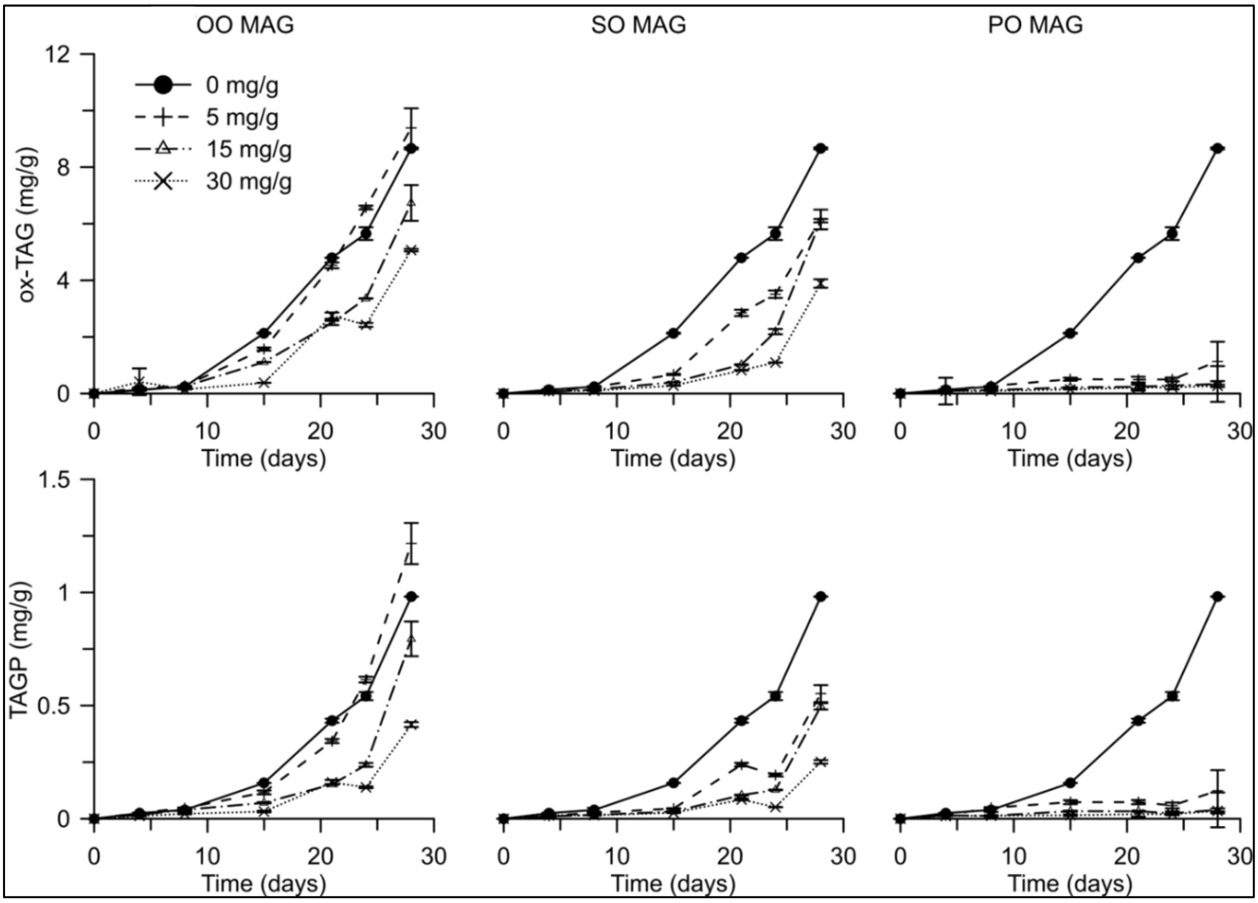

Figure 3 - Determination of oxidized triacylglycerols (ox-TAG) and triacylglycerol oligopolymers (TAGP) in purified olive oil and in the same added with 0.5%, 1.5%, and 3% of monoacylglycerols (MAG-5 mg/g, MAG-15 mg/g, MAG-30 mg/g) of olive oil (OO), soybean oil (SO), and palm oil (PO) during the oven test at 60°C.

increase in the antioxidant effect; this point provides evidence that differences in the MAG fatty acid composition could act differently in modulating the oxidation process.

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