

## The effect of rosemary extract on 3-MCPD and glycidyl esters during frying

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**SUMMARY:** The aim of the present work was to evaluate the effect of rosemary extract (*Rosmarinus Officinalis* L.) on the 3-MCPD and glycidyl ester contents during deep-fat frying. Firstly, the potato samples were soaked in different concentrations of NaCl and they were fried in sunflower oils containing different concentrations of rosemary extract. Subsequently, repeated frying was carried out with sunflower oil enriched with rosemary extract. The frying oil and the oil extracted from potato samples were analyzed in terms of the contents of 3-MCPD and glycidyl esters according to DGF C VI 18 (10) method. The results demonstrated that the content of 3-MCPD and glycidyl esters in frying oil was not significantly affected by different NaCl and rosemary extract concentrations during short term frying; however, the amount of 3-MCPD esters was found to be lower for the potato crisps enriched with rosemary extract during repeated frying cycles.

**KEYWORDS:** 3-MCPD esters; Deep-fat frying; Glycidyl esters; Rosemary extract; Sunflower oil

**RESUMEN:** Efecto de extractos de romero sobre 3-MCPD y ésteres de glicidol durante frituras. El objetivo del presente trabajo fue evaluar el efecto del extracto de romero (*Rosmarinus Officinalis* L.) sobre los contenidos de 3-MCPD y ésteres de glicidol durante la fritura. En primer lugar, muestras de patatas se remojaron en diferentes concentraciones de NaCl y se frieron en aceites de girasol que contienen diferentes concentraciones de extracto de romero. Posteriormente, se realizaron frituras repetidas con aceite de girasol enriquecido con extracto de romero. El aceite de fritura y el aceite extraído de las muestras de patatas se analizaron en términos de los contenidos de 3-MCPD y ésteres de glicidol de acuerdo con el método DGF C VI 18. Los resultados demostraron que el contenido de 3-MCPD y ésteres de glicidol en el aceite de fritura no se vieron afectados significativamente por las diferentes concentraciones de NaCl a corto plazo, sin embargo, la cantidad de ésteres de 3-MCPD fue menor para las patatas fritas enriquecidas con extracto de romero durante los ciclos de fritura repetidas.

**PALABRAS CLAVE:** 3-MCPD ésteres; Aceite de girasol; Ésteres de glicidol; Extracto de romero; Fritura

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## 1. INTRODUCTION

Fatty acid esters of 3-monochloro-propane-1,2-diol (3-MCPD) and glycidol are known as food processing contaminants which were discovered years ago in oils and oil-containing food products. 3-MCPD esters are formed when lipids react with chlorine containing compounds at elevated temperatures (Hrcirik and Duijn, 2011). Significant amounts of 3-MCPD and glycidyl esters have been detected in refined vegetable oils, since the deodorization step of refining requires temperatures of between 240 °C and 270 °C, depending on the type of oil (Bockisch, 1993).

The maximum daily tolerable limit was established as 2 µg/kg body weight for free 3-MCPD (JECFA, 2001). 3-MCPD was reported to be separated from its esters in the gastrointestinal system (Bockisch, 1993). Hence, the defined limit for daily consumption in its free form was reported to be valid for the ester form. In acute studies, 3-MCPD toxicologically affected kidneys, blood, and sperm and in long-term studies, 3-MCPD led to the formation of tumors in rats (Matthäus *et al.*, 2011). Glycidol, formed by the decomposition of glycidyl esters, is known as a probable carcinogen for humans (IARC, 2000).

Deep fat frying is a very common process used to prepare various food products with a pleasant flavor. During frying, many volatile and non-volatile compounds (Choe and Min, 2007), some of which may be toxic, are formed due to hydrolytic, thermal and oxidative reactions because the oil is exposed to high temperatures in the presence of water and air. The degradation products of frying oil, including diacylglycerols have been reported to cause the formation of glycidyl (Aniołowska and Kita, 2016) and 3-MCPD esters (Arisseto *et al.*, 2017). It was also stated that during the frying process, the components in the frying medium may be absorbed by the fried food (Aniołowska and Kita, 2015). Different approaches, including utilization of both natural and artificial antioxidants and other additives, have been developed to prevent undesirable changes and to improve the quality and stability of frying oil (Chammem *et al.*, 2015). The fortification of refined oils with antioxidants is commonly implemented by oil manufacturers since the antioxidants delay the undesirable changes during storage and frying operations and hence prolong shelf life of fried goods (Man and Jaswir, 2000). The use of artificial antioxidants has been limited in some countries due to possible adverse health effects (Chen *et al.*, 1992). Therefore, plant-derived natural antioxidants are gaining increasing interest. Rosemary (*Rosmarinus officinalis* L.) extract is the most attractive natural antioxidant due to its high thermal resistance (Man and Jaswir, 2000) and protective effect during frying (Man and Jaswir, 2000; Filip *et al.*, 2011). Oleoresin rosemary has been determined to eliminate thermooxidative changes during

frying (Upadhyay *et al.*, 2017a). Similarly, the synergistic effect of oleoresin sage and ascorbyl palmitate has been reported to slow down the thermooxidation of sunflower oil (Upadhyay *et al.*, 2016; Sehwag *et al.*, 2018). Recently, electronic noise guidance has been used to determine the appropriate frying disposal time (Upadhyay *et al.*, 2017b).

Until today, most of the research about the formation of 3-MCPD and glycidyl esters was focused on refining stages, mainly the deodorization step. However, the occurrence of these contaminants during deep-fat frying is also worth researching, since their formation mechanism is still hardly known. Moreover, the effect of different additives on the formation of 3-MCPD and glycidyl esters was restrictively researched. Zhang *et al.*, (2016) investigated the possible mechanism of free radical scavengers, including L-ascorbyl palmitate,  $\alpha$ -tocopherol, lipophilic tea polyphenols and rosemary extract on the mitigation of 3-MCPD ester formation in vegetable oils and stated that all of the antioxidants decreased 3-MCPD esters in palm oil during deodorization. Li *et al.*, (2015) proposed butylated hydroxytoluene, butylated hydroxy anisole, tert-butyl hydroquinone, propyl galate, L-ascorbyl palmitate and  $\alpha$ -tocopherol as potential inhibitors of 3-MCPD esters in practice. Because the consumption of fried food products can represent an important route for human exposure to 3-MCPD (EFSA, 2016; Arisseto *et al.*, 2017), the factors which are effective on their formation have to be deeply researched to develop effective mitigation strategies in order to reduce their daily intake. In this sense, this research was focused on the evaluation of the effect of rosemary extract on 3-MCPD and glycidyl esters formed during the deep-fat frying of potato crisps, also taking into account other compositional changes.

## 2. MATERIALS AND METHODS

### 2.1. Materials and chemicals

Sunflower oil, potatoes and dried rosemary leaves were purchased from a local market. 3-chloro-1,2-propanediol, 3-chloro-1,2-propanediol-d5, glycidol, hexane, isooctane, tert-butyl methyl ether, diethyl ether, ethyl acetate, sodium methoxide, toluene, phenyl boronic acid, hydrochloric acid, methanol, *p*-anisidine, sodium hydroxide were purchased from Sigma-Aldrich (St- Louis, ABD). Sodium chloride, sodium bromide, potassium hydroxide and phenolphthalein were bought from Merck (Darmstadt, Germany).

### 2.2. Methods

**Preparation of rosemary extract.** The rosemary extract was obtained from ground rosemary leaves according to the Soxhlet method. Dried rosemary

leaves of 50 g were weighed and placed in a soxhlet apparatus. Extraction was carried out with ethyl alcohol (96%) for 2 hours. A rotary vacuum evaporator at 50 °C was used to evaporate the solvent. The residual extract was washed using 60 ml of ethyl alcohol for 3 times and the solvent was removed under nitrogen atmosphere. The extract was kept in dark bottles at -18 °C until analysis.

**Deep-fat frying.** The frying experiments were conducted in two steps. In the first step, potato samples were deep-fried to investigate the effects of different salt and rosemary extract concentrations. The potatoes were sliced to a thickness of 2 mm × 40 mm and then they were kept submerged in different concentrations (0, 1, 2.5 and 5%) of sodium chloride solutions. The rosemary extract was added to frying oil at different levels (0, 500, 1000 ve 2000 mg/kg) just before frying. A batch of 50 g of potatoes was fried in 1.5 kg of refined sunflower oil in an electrical fryer (Remta, Turkey) for 3 minutes at 180 °C. There were a total of 16 frying systems and the oil was not reused in any process. Each experiment was performed in duplicate. The oil used for the frying process and the oil extracted from potatoes were analyzed for their 3-MCPD and glycidyl ester content.

In the second step, repeated frying was carried out for 3 consecutive days for 8 hours daily with the same frying parameters (180 °C for 3 minutes). Before repeated frying cycles, the potato samples were soaked in distilled water for 1 hour prior to frying and subsequently fried in sunflower oil fortified with 2000 mg/kg of rosemary extract. At the end of each frying day, the fryers were shut off and the oils were filtered through filter paper to remove residuals and kept at 4 °C until the following day. The oil was replenished with rosemary-extract-added (2000 mg/kg) oil each day before frying. Both the fourth and eighth batches of potato chips and 50 ml of frying oil samples were taken each day and kept at -18 °C until analysis.

**The extraction of oil from potatoes.** The oil absorbed by potatoes during frying was extracted using a semi-automatic solvent extractor (SER148, Velp Scientifica, Italy). Hexane was used as solvent and extracted oils were stored at -18°C until analysis.

**Determination of 3-MCPD and glycidyl ester contents.** 3-MCPD and glycidyl ester analysis was carried out according to the DGF method C-VI 18 (10) (DGF, 2010) which is based on alkaline transesterification. Quantification was performed according to the method of Cheng *et al.*, (2016) in a Shimadzu QP2020 system (Shimadzu, Kyoto, Japan) equipped with HP-5MS capillary column (0.32 mm × 0.25 µm, Agilent Technologies, USA). Samples were injected in splitless mode. The oven temperature was programmed from 80 °C to 155 °C at a rate of 5 °C/min and increased to 300 °C at a rate of 15 °C/min, and

held for 5 min. Helium was used as carrier gas at a flow rate of 1.18 ml/min. The mass spectrometer was operated in selected ion monitoring mode with positive electron ionization (EI+) at an ionization voltage of 70 eV. The temperature of the ion source and interface in the mass spectrometer was 200 and 280 °C, respectively. The ion traces m/z 147 and 150 were selected as the quantitative ions for the quantitation of 3-MCPD and 3-MCPD-d5, respectively.

**Fatty acid composition.** The percentage of fatty acids in frying oils was determined by capillary gas chromatography (GC 2010, Shimadzu, Kyoto, Japan) on fatty acid methyl esters obtained according to IUPAC (1987). Chromatographic separation was achieved using a DB-23 fused silica capillary column (60 m × 0.25 mm internal diameter × 0.25 µm film thickness, J&W Scientific). The split ratio was 80:1. The temperatures of the column, injector and detector were 195 °C, 230 °C and 240 °C, respectively.

**Statistical analysis.** Statistical analysis was carried out using SPSS 15 statistical software (SPSS Inc., Chicago, USA). Data were evaluated by the one-way ANOVA procedure using the Duncan's multiple range test. A *p*-value of less than 0.05 was considered significant.

### 3. RESULTS AND DISCUSSION

#### 3.1. 3-MCPD and glycidyl ester contents of frying oils and potato crisps

Deep-fat frying is one of the most widely used processes in the world for food processing. In the current study, before the frying process, the potatoes were soaked in different concentrations of sodium chloride (NaCl), since chlorine-containing compounds are known to be the potential precursors in the formation of 3-MPCD esters (Dingel and Matissek, 2015). NaCl was chosen as the chlorine-containing compound because it is one of the favorite seasoning and preservative agents in the food industry (Wong *et al.*, 2017). Antioxidants have been reported to have the ability to eliminate the precursors of 3-MCPD esters such as cyclic acyloxonium free radical (CAFR) intermediates and chlorine radicals in a previously published work (Zhang *et al.*, 2016). Hence, rosemary extract was selected as the antioxidant source because it has been widely used in foods and approved as a GRAS (Generally recognized as safe) food additive.

The influence of NaCl and rosemary extract on the content of 3-MCPD esters in both frying oil and potato crisps is presented in Table 1. The total amount of 3-MCPD esters in frying oils ranged from 0.52 to 0.96 mg/kg. The addition of rosemary extract did not affect the concentration of 3-MCPD esters in frying oil significantly. The influence of

TABLE 1. The effect of rosemary extract and NaCl on 3-MCPD ester contents in frying oil and potato crisps (mg/kg)

NaCl (%)	Rosemary extract (mg/kg)			
	0	500	1000	2000
<i>Frying Oil</i>				
0	0.57	0.96	0.73	0.80
1	0.52	0.70	0.58	0.76
2.5	0.90	0.76	0.72	0.71
5	0.79	0.78	0.53	0.77
<i>Potato Crisps</i>				
0	0.83 <sup>a,AB</sup>	0.81 <sup>a,A</sup>	1.08 <sup>a,AB</sup>	1.01 <sup>a,A</sup>
1	0.78 <sup>ab,AB</sup>	0.95 <sup>ab,A</sup>	0.65 <sup>a,AB</sup>	1.46 <sup>b,A</sup>
2.5	0.71 <sup>a,A</sup>	0.85 <sup>a,A</sup>	1.21 <sup>a,B</sup>	0.71 <sup>a,A</sup>
5	1.19 <sup>b,B</sup>	1.23 <sup>b,A</sup>	0.36 <sup>a,A</sup>	1.43 <sup>b,A</sup>

Results are reported as mean value of four replicate analyses. Different lowercase letters show differences in the same line, whereas uppercase letters indicate differences in the same column (Duncan test,  $p < 0.05$ ).

sodium chloride was also statistically insignificant on the content of bound 3-MCPD. However, the same tendency was not observed for potato crisps. The effects of both the NaCl and rosemary extract concentration were found to be statistically significant on the 3-MCPD ester contents of potatoes. The highest level of 3-MCPD esters was determined as 1.46 mg/kg; whereas the lowest level was detected to be 0.36 mg/kg in crisps. The changes in NaCl concentration were found to be statistically significant when the samples were fortified with 1000 mg/kg rosemary extract compared to when rosemary extract was not added prior to frying.

The results on the effect of NaCl and rosemary extract on the content of glycidyl esters are presented in Table 2. The glycidyl ester contents vary between 0.19 and 0.48 mg/kg for frying oils. The differences between the different concentrations of NaCl were not significant during deep-fat frying, as was stated by Wong *et al.* (2017). The content of glycidyl esters in potato crisps ranged from 0.15 and 0.41 mg/kg. The results given in Table 2 indicate that both NaCl and rosemary extract were not influential on the content of glycidyl esters. The content of glycidyl esters were generally found to be lower than 3-MCPD esters for both frying oils and oils extracted from potatoes. The non-formation of glycidyl esters can be attributed to the low frying temperature in accordance with different studies in the literature which point out that the content of glycidyl esters increases dramatically when the temperature is higher than 230 °C (Zelinková *et al.*, 2006; Matthäus and Pudiel, 2015).

Table 3 shows the changes in 3-MCPD and glycidyl ester levels during repeated frying at seven different points in time: 0, 4, 8, 12, 16, 20 and 24 hours. The content of ester-bound 3-MCPD of sunflower oil (SO) ranged from 0.42 and 1.01 mg/kg; whereas

TABLE 2. The effect of rosemary extract and NaCl on glycidyl ester contents in frying oil and potato crisps (mg/kg)

NaCl (%)	Rosemary extract (mg/kg)			
	0	500	1000	2000
<i>Frying Oil</i>				
0	0.39	0.20	0.29	0.22
1	0.27	0.36	0.47	0.24
2.5	0.21	0.33	0.27	0.21
5	0.48	0.19	0.36	0.39
<i>Potato Crisps</i>				
0	0.29	0.33	0.24	0.22
1	0.15	0.25	0.31	0.30
2.5	0.18	0.18	0.21	0.21
5	0.21	0.26	0.41	0.26

Results are reported as mean value of four replicate analyses. Different lowercase letters show differences in the same line, whereas uppercase letters indicate differences in the same column (Duncan test,  $p < 0.05$ ).

it was between 0.42 and 0.79 mg/kg for sunflower oil enriched with rosemary extract (SOR). There was no statistically significant change in the content of 3-MCPD esters of either SO or SOR as the frying time increased. The changes in the concentration of ester-linked 3-MCPD esters detected in the oil samples extracted from potatoes are presented in Table 3 in order to evaluate the carry-over of the compounds due to fat uptake. The amount of 3-MCPD esters was in the range of 0.56-0.94 mg/kg for SO, while it was in the range of 0.19-0.64 mg/kg for SOR. The content of 3-MCPD esters was mainly lower for SOR, which may point to the inhibitory effect of rosemary extract on 3-MCPD levels during repeated frying. Generally, it can be noted that 3-MCPD esters were transferred from the frying medium to the fried product in accordance with the results of Ariseto *et al.*, (2017) who found that 3-MCPD esters have the ability to be transferred from frying oil to fried material during the deep-fat frying of various food stuffs.

The content of glycidyl esters in the frying oils were in the range of 0.06-0.37 mg/kg for SO and 0.07-0.37 mg/kg for SOR. The concentration of glycidyl esters in SO changed significantly with frying time. At the end of 8, 12, 20, and 24 hours of frying, the glycidyl ester contents in SOR were lower than SO. Additionally, the increase in glycidyl esters at 8, 12 and 24 hours was statistically significant for SO. Wong *et al.*, (2017) reported the trend for glycidyl esters to increase during prolonged frying. However, there are also contradictory reports which have shown a decrease in the concentration

TABLE 3. The effect of rosemary extract and NaCl on 3-MCPD and glycidyl ester contents in frying oil and potato crisps during repeated frying ( $p < 0.05$ ).

Frying time (h)	3-MCPD esters (mg/kg)		Glycidyl Esters (mg/kg)	
	SO	SOR	SO	SOR
<i>Frying Oil</i>				
0	0.59	0.59	0.37 <sup>A,x</sup>	0.37 <sup>A,x</sup>
4	0.98	0.64	0.06 <sup>A,x</sup>	0.11 <sup>A,x</sup>
8	0.80	0.65	0.27 <sup>CD,y</sup>	0.12 <sup>A,x</sup>
12	1.01	0.79	0.31 <sup>DE,y</sup>	0.07 <sup>A,x</sup>
16	0.53	0.73	0.06 <sup>A,x</sup>	0.07 <sup>A,x</sup>
20	0.42	0.42	0.14 <sup>B,x</sup>	0.12 <sup>A,x</sup>
24	0.82	0.56	0.20 <sup>BC,y</sup>	0.07 <sup>A,x</sup>
<i>Potato Crisps</i>				
4	0.89 <sup>y</sup>	0.59 <sup>x</sup>	0.19 <sup>A,x</sup>	0.14 <sup>x</sup>
8	0.80 <sup>x</sup>	0.64 <sup>x</sup>	0.19 <sup>A,x</sup>	0.13 <sup>x</sup>
12	0.75 <sup>x</sup>	0.56 <sup>x</sup>	0.13 <sup>A,x</sup>	0.14 <sup>x</sup>
16	0.94 <sup>x</sup>	0.60 <sup>x</sup>	0.07 <sup>A,x</sup>	0.16 <sup>x</sup>
20	0.56 <sup>x</sup>	0.53 <sup>x</sup>	0.16 <sup>A,x</sup>	0.34 <sup>x</sup>
24	0.83 <sup>x</sup>	0.19 <sup>x</sup>	0.45 <sup>B,y</sup>	0.16 <sup>x</sup>

Results are reported as mean value of four replicate analyses. Different uppercase letters indicate differences in the same column, whereas “x-y” show the differences between SO and SOR (Duncan test,  $p < 0.05$ ), SO: sunflower oil, SOR: sunflower oil containing rosemary extract

of glycidyl esters during prolonged deep-fat frying (Aniołowska and Kita, 2015; Hammouda *et al.*, 2017; Aniołowska and Kita, 2016). In general, the concentration of glycidyl esters in potato crisps did

not change significantly when rosemary extract was added to the frying oil.

### 3.2. Fatty acid composition of frying oils

The changes in fatty the acid composition of the frying oil during repeated frying are given in Table 4. Sunflower oil showed a typical fatty acid composition including 56.89% linoleic, 34.03% oleic, 5.97% palmitic and 2.91% stearic acids as is common for most sunflower oils (Sánchez-Gimeno *et al.*, 2008; Filip *et al.*, 2011). There was an obvious reduction in linoleic acid, with a consequent increase in the contents of oleic acid and palmitic acids as reported by Romero *et al.*, (2000) and Casal *et al.*, (2010). The content of linolenic acid decreased with frying time. There was not a significant difference between sunflower and rosemary extract containing sunflower oil in terms of fatty acid distribution as was also previously reported by Filip *et al.*, (2011).

## 4. CONCLUSIONS

The results of the study show that the effects of sodium chloride and fortification of frying oil with rosemary extract were statistically insignificant on the formation of 3-MCPD and glycidyl esters in frying oil during the deep-fat frying of potato crisps. However the bound 3-MCPD content in the oil extraction showed statistically significant alterations. The concentration of glycidyl esters was always lower than 3-MCPD esters throughout the frying process. In addition, the amount of 3-MCPD esters was higher in the potato oils, while the amount of glycidyl esters was generally higher in the frying oils. With regards

TABLE 4. The changes in fatty acid composition of frying oils during repeated frying (%)

Frying time (h)	Oil type	Fatty acid composition (%)							
		C 16:0	C 16:1	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 18:3
0	SO	5.97 <sup>A,x</sup>	0.13 <sup>AB,x</sup>	0.01 <sup>A,x</sup>	0.01 <sup>A,x</sup>	2.91 <sup>B,x</sup>	34.03 <sup>A,x</sup>	56.89 <sup>C,x</sup>	0.05 <sup>A,x</sup>
	SOR	5.97 <sup>a,x</sup>	0.13 <sup>b,x</sup>	0.01 <sup>a,x</sup>	0.01 <sup>a,x</sup>	2.91 <sup>ab,x</sup>	34.03 <sup>a,x</sup>	56.89 <sup>ex</sup>	0.05 <sup>a,x</sup>
4	SO	6.01 <sup>A,x</sup>	0.08 <sup>A,x</sup>	0.01 <sup>A,x</sup>	0.01 <sup>A,x</sup>	2.85 <sup>AB,x</sup>	34.87 <sup>B,x</sup>	56.15 <sup>C,x</sup>	0.05 <sup>A,x</sup>
	SOR	6.18 <sup>b,x</sup>	0.14 <sup>bc,y</sup>	0.02 <sup>ab,y</sup>	0.01 <sup>a,x</sup>	3.00 <sup>ab,x</sup>	34.68 <sup>b,x</sup>	55.93 <sup>d,x</sup>	0.04 <sup>a,x</sup>
8	SO	6.40 <sup>B,x</sup>	0.07 <sup>A,x</sup>	0.09 <sup>A,x</sup>	0.10 <sup>A,x</sup>	2.75 <sup>A,x</sup>	35.91 <sup>C,x</sup>	55.82 <sup>C,x</sup>	0.04 <sup>A,x</sup>
	SOR	6.27 <sup>b,x</sup>	0.08 <sup>ab,x</sup>	0.02 <sup>ab,x</sup>	0.01 <sup>A,x</sup>	2.82 <sup>a,x</sup>	36.08 <sup>c,x</sup>	54.69 <sup>c,x</sup>	0.04 <sup>a,x</sup>
12	SO	6.53 <sup>BC,x</sup>	0.12 <sup>AB,y</sup>	0.03 <sup>A,x</sup>	0.02 <sup>A,x</sup>	3.25 <sup>C,x</sup>	36.24 <sup>C,x</sup>	53.79 <sup>B,x</sup>	0.04 <sup>A,x</sup>
	SOR	6.32 <sup>b,y</sup>	0.05 <sup>a,x</sup>	0.01 <sup>a,x</sup>	0.01 <sup>a,x</sup>	3.05 <sup>ab,x</sup>	36.17 <sup>c,x</sup>	54.35 <sup>c,y</sup>	0.04 <sup>a,x</sup>
16	SO	6.76 <sup>CD,x</sup>	0.16 <sup>B,y</sup>	0.11 <sup>A,x</sup>	0.12 <sup>A,x</sup>	3.28 <sup>C,x</sup>	36.91 <sup>D,x</sup>	52.80 <sup>B,x</sup>	0.24 <sup>A,x</sup>
	SOR	6.60 <sup>c,x</sup>	0.11 <sup>bc,x</sup>	0.02 <sup>ab,x</sup>	0.01 <sup>a,x</sup>	3.22 <sup>ab,x</sup>	37.14 <sup>d,y</sup>	52.87 <sup>b,x</sup>	0.09 <sup>a,x</sup>
20	SO	6.78 <sup>CD,x</sup>	0.12 <sup>AB,x</sup>	0.03 <sup>A,x</sup>	0.02 <sup>A,y</sup>	3.12 <sup>BC,x</sup>	37.25 <sup>D,x</sup>	52.68 <sup>B,x</sup>	0.03 <sup>A,x</sup>
	SOR	6.68 <sup>c,x</sup>	0.16 <sup>c,y</sup>	0.02 <sup>ab,x</sup>	0.01 <sup>a,x</sup>	2.83 <sup>a,x</sup>	37.31 <sup>d,x</sup>	52.71 <sup>b,x</sup>	0.03 <sup>a,x</sup>
24	SO	6.95 <sup>D,x</sup>	0.18 <sup>B,y</sup>	0.02 <sup>A,x</sup>	0.01 <sup>A,x</sup>	3.35 <sup>C,x</sup>	38.28 <sup>C,x</sup>	51.17 <sup>A,x</sup>	0.04 <sup>A,y</sup>
	SOR	6.75 <sup>c,x</sup>	0.11 <sup>bc,x</sup>	0.02 <sup>b,x</sup>	0.01 <sup>a,x</sup>	3.25 <sup>b,x</sup>	38.28 <sup>ex</sup>	51.54 <sup>ay</sup>	0.03 <sup>a,x</sup>

Results are reported as mean value of four replicate analyses.

The different uppercase letters (A-D) show differences for sunflower oil (SO); lowercase letters (a-e) indicate differences for sunflower oil enriched with rosemary extract (SOR); and lowercase letters (x-y) indicate differences between SO and SOR in the same line during repeated frying (Duncan test,  $p < 0.05$ ).

to repeated frying, the content of 3-MCPD esters was in general higher in non-fortified samples. Moreover, during repeated frying, the maximum levels of 3-MCPD and glycidyl ester contents were detected in the oil samples which were not fortified with rosemary extract when compared to enriched oils. From this point of view, the results allow for the assertion that the rosemary extract might have a potential to minimize the formation of 3-MCPD and glycidyl esters under repeated frying conditions at certain frying times. Considering potato oils during repeated frying, on the other hand, the frying duration was generally not influential on the formation of 3-MCPD and glycidyl esters. However the content of 3-MCPD esters was always lower in oil samples fortified with rosemary extract. Considering the scarce reports on the effect of different antioxidants on the occurrence of 3-MCPD and glycidyl esters in the literature, the current data reported herein may be useful to fill the gap in the literature. Further studies can focus on researching the effects of novel antioxidants from various sources on 3-MCPD and glycidyl ester formation and the mechanisms of antioxidants on the 3-MCPD and glycidyl ester contents should also be clarified.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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