Sesame oil inhibits the formation of glycidyl ester during deodorization

Lipeng Han\textsuperscript{a}, Jiahui Li\textsuperscript{b}, Shujie Wang\textsuperscript{b}, Weiwei Cheng\textsuperscript{c}, Lukai Ma\textsuperscript{d}, Guoqin Liu\textsuperscript{b*}, Dongxue Han\textsuperscript{a*}, and Li Niu\textsuperscript{a}

\textsuperscript{a}School of Chemistry and Chemical Engineering, Guangzhou University, Guangzhou, China; \textsuperscript{b}School of Food Science and Engineering, South China University of Technology, Guangzhou, China; \textsuperscript{c}Institute for Advanced Study, Shenzhen University, Shenzhen, China; \textsuperscript{d}College of Light Industry and Food, Zhongkai University of Agriculture and Engineering, Guangzhou, China

**ABSTRACT**

Glycidyl ester (GE) has attracted worldwide attention due to its potential harm to human health. The GE in edible oil forms during the deodorization process of the oil refining, where antioxidants are used to inhibit its formation. To replace unsafe conventional synthetic antioxidants with natural antioxidants, sesame oil (SO) was used to inhibit the formation of GE in corn oil (CO), palm oil (PO), and rice bran oil (RO) during the deodorization process. The results showed that the content of GE in SO, CO, PO and RO followed the decreasing order of RO (1395.88 µg/kg) > PO (376.84 µg/kg) > CO (303.24 µg/kg) > SO (133.19 µg/kg). The concentration of diacylglycerol (DAG) in SO, CO, PO and RO followed the decreasing order of RO (11.64%) > PO (7.56%) > CO (4.11%) > SO (2.59%). The content of GE in oil could be affected by at least three factors: (i) unsaturated fatty acids could promote the formation of GE in oil; (ii) antioxidants in oil could inhibit the formation of GE by scavenging free radicals; and (iii) DAG, as a precursor, could lead to the formation of GE. This result suggested that SO could inhibit the formation of GE in CO, PO and RO. This indicated that when SO was added at 50%, its inhibitory effect was equivalent to that of tert-butylhydroquinone (TBHQ) added at 0.02%.

**Introduction**

Glycidyl ester (GE) has attracted worldwide attention due to its potential harm to human health.\textsuperscript{[1–6]} GE is the esterification product of glycidol and fatty acid. After consumption, GE is digested in the gastrointestinal tract, leading to systemic exposure to the reactive epoxide glycidol.\textsuperscript{[7]} The epoxide glycidol is carcinogenic, genotoxic and teratogenic in rodents and is rated by the International Agency for Research on Cancer (IARC) as “probably carcinogenic to humans” (Group 2A).\textsuperscript{[8]}

The GE in oil mainly forms during the deodorization process of oil refining.\textsuperscript{[9]} Chew et al. studied the variation of GE content in kenaf seed oil during the oil refining. The results showed that no GE was detected in crude, degummed, neutralized, and bleached oil. The GE content in deodorized oil reached 54.8 µg/kg.\textsuperscript{[10]}

Adding antioxidant is a simple, economical and effective method to inhibit the formation of GE during the deodorization process. We investigated the inhibitory effect of tert-butylhydroquinone (TBHQ) on the formation of GE. The results showed that when the mass percentage of TBHQ added was 0.05%, the inhibition percentages of TBHQ on GE in model palm oil and model corn oil were

**CONTACT** Guoqin Liu (guoqin@sut.edu.cn) School of Food Science and Engineering, South China University of Technology, Wushan Road 381, Tianhe District, Guangzhou, China; Dongxue Han (dxhan@gzhu.edu.cn) School of Chemistry and Chemical Engineering, Guangzhou University, Waihuan Xi Road 230, Panyu District, Guangzhou, China

*These authors contributed equally.

© 2021 Lipeng Han, Jiahui Li, Shujie Wang, Weiwei Cheng, Lukai Ma, Guoqin Liu, Dongxue Han and Li Niu. Published with license by Taylor & Francis Group, LLC. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
37.5% and 25.0%, respectively.\textsuperscript{[11]} TBHQ can inhibit the formation of GE. Synthetic antioxidants, such as TBHQ, have been commonly used as food antioxidants to prevent oil oxidation due to their ability to scavenge free radicals. However, extensive studies have demonstrated that TBHQ exhibits a carcinogenic effect.\textsuperscript{[12]} In North America, the maximum content of TBHQ allowed in oil products is 0.02%, with an acceptable daily intake of 0–0.7 mg/kg body weight.\textsuperscript{[13]} Food scientists thus attempt to replace synthetic antioxidants with natural products. Wong et al. compared the inhibitory effects of oleoresin rosemary, sage extract, TBHQ, butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) on the content of GE in frying oil. The results showed that when heated at 180°C for 3 days, with the mass percentage of added antioxidant of 0.02%, the loss of the content of GE in frying oil followed the order of TBHQ (32.9%) > oleoresin rosemary (30.5%) > sage extract (26.5%) > BHA (26.2%) > BHT (23.5%). This suggested that oleoresin rosemary had a strong inhibitory effect on the formation of GE.\textsuperscript{[14]} However, oleoresin rosemary is hydrophilic. We attempt to find natural products that preferably originated from oil so that we can prepare oil blends.

Sesame oil (SO) has outstanding antioxidant activity because of its lignans and tocopherols. Lignans such as sesamin, sesamolin, and sesamol are present in SO at approximately 6.5–17.3 g/kg. α-Tocopherol, β-tocopherol and δ-tocopherol levels in SO are approximately 56.9–99.3, 0.034–0.175 and 0.44–3.05 mg/kg, respectively.\textsuperscript{[15]} Therefore, we speculate that SO can inhibit the formation of GE due to its natural antioxidants.

The aim of this work is to investigate the inhibitory effect of SO on the formation of GE during the deodorization process. Our work will contribute to the final control of GE with SO.

**Materials and methods**

**Reagents, materials, and apparatus**

Reagents. 3-Monochloropropane-1,2-diol (3-MCPD, purity 99%), isotopically labeled 3-monochloropropane-1,2-diol (d5-3-MCPD, purity 99%) and TBHQ (purity 98%) were purchased from Aladdin Reagent Co., Ltd (China, Shanghai). Phenylboronic acid, methyl tertiary butyl ether, sodium methoxide, sodium chloride, sodium bromide, methanol, hexane, potassium hydroxide, ethyl acetate, and anhydrous sodium sulfate were of analytical grade, and they were purchased from Comeo Reagent Co., Ltd (China, Tianjin).

Materials. Refined corn oil (CO), palm oil (PO), and rice bran oil (RO) were all offered by Yihai Co., Ltd. (China, Shenzhen) without added synthetic antioxidants. Sesame seeds were purchased from the local supermarket. Silica gel (100–200 mesh) was acquired from BANKE Separation Materials Co., Ltd (China, Qingdao).

Apparatus. Gas chromatography-mass spectrometry (GC-MS, Agilent 6890 N-5975B, USA). High-performance liquid chromatography (HPLC, Agilent 1260, U.S.A.). Refractive index detector (RID, Agilent G7162A, U.S.A.).

**Preparation of oil**

The SO was performed according to Cheng et al.\textsuperscript{[16]} Washed and dried sesame seeds were ground and steamed over boiling water for 5 min, then finally pressed to produce SO. The SO was stored at 4°C. The CO, PO and RO were treated according to Cheng et al.\textsuperscript{[11]} to removed their GE and DAG.

**Analysis of fatty acid composition of oil before deodorization**

The method was performed according to Han et al.\textsuperscript{[17]} A total of 0.06 g oil was dissolved in 4 mL hexane, respectively. A total of 0.2 mL of 2 mol/L potassium hydroxide-methanol solution was added, and the mixture was stirred for 30 min. The supernatant was added with anhydrous sodium sulfate, passed through a membrane filter (0.22 μm) and detected by GC-MS. Chromatographic conditions: an
Agilent TG-5 MS capillary column (30 m × 0.25 mm × 0.25 μm) was used at an oven temperature of 130°C for 1 min, which was then increased to 220°C at 6°C/min and held for 5 min. The injection volume was 0.2 μL, the sample temperature was 200°C, and the carrier gas was helium with a flow rate of 1 mL/min. The hierarchical cluster analysis module of ChemStation was used to analyze the fatty acid compositions of five kinds of oil.

The fatty acid composition of oil was qualitatively determined by area normalization. The number of moles of carbon-carbon unsaturated double bonds in oil per kilogram (N_{C=C}) was calculated as follows:

\[ N_{C=C} = 1 \times \frac{\text{mass percentage of oleic acid}}{\text{mol ar mass of oleic acid}} + 2 \times \frac{\text{mass percentage of linoleic acid}}{\text{mol ar mass of linoleic acid}} + 3 \times \frac{\text{mass percentage of linolenic acid}}{\text{mol ar mass of linolenic acid}} \]

The molar mass of oleic acid was 282 g/mol, linoleic acid was 280 g/mol, and linolenic acid was 278 g/mol.

**The inhibitory effect of sesame oil on the formation of glycidyl ester and diacylglycerol in the laboratory-scale refining process**

The method was performed according to Cheng et al.\[18\] A total of 500.0 g of CO, PO, or RO was added with a certain mass percentage of SO (10%, 20%, 30%, 40%, or 50%) or TBHQ (0.02%), and then deodorized at 200°C for 1 h. A constant steam strip with the speed of 0.15 mL/min was continuously passed through the bottom. The deodorizer was equipped with a three-necked flask and a thermally controlled heating mantle. The three necks were connected with an electric thermometer from the heating mantle, an inlet tube from the steam generator and a vacuum pump, respectively. Pure CO, PO, RO, or SO were also deodorized by the same method as the blank samples. The deodorized samples were stored at −18°C until analysis.

The calculated content of GEs in CO, PO or RO added with TBHQ is defined as the content of GEs in pure CO, PO or RO. The experimental content of GEs in CO, PO or RO added with SO or TBHQ is defined as the content of GEs in CO, PO or RO added with SO or TBHQ. The experimental concentration of DAG in CO, PO or RO added with SO is defined as the concentration of DAG in CO, PO or RO added with SO. Other relevant indicators are calculated according to the following formulas:

\[
\text{Calculated content of GEs in CO, PO or RO added with SO (μg/kg)} = \frac{\text{content of GEs in pure CO, PO or RO} + \text{content of GEs in pure SO} \times \text{percentage of SO added}}{1 + \text{percentage of SO added}}
\]

\[
\text{Inhibition percentage of SO on GEs in CO, PO or RO added with SO or TBHQ} = \frac{\text{Decreased content of GEs in CO, PO or RO added with SO or TBHQ}}{\text{content of GEs in pure CO, PO or RO}} \times 100\%
\]

\[
\text{Concentration of DAG} = \frac{\text{content of DAG}}{\text{content of TAG} + \text{content of FFA} + \text{content of DAG} + \text{content of MAG}} \times 100\%
\]
Calculated concentration of DAG in CO, PO or RO added with SO\(^{\%}\) 
\[
\frac{\text{concentration of DAG in pure CO, PO or RO} + \text{concentration of DAG in pure SO} \times \text{percentage of SO added}}{1 + \text{percentage of SO added}}
\]

Decreased concentration of DAG in CO, PO or RO added with SO\(^{\%}\) 
\[
= \text{Calculated concentration of DAG added with SO} - \text{Experiment concentration of DAG added with SO}
\]

**Determination of the content of glycidyl ester in oil after deodorization**

The method was performed according to Cheng et al.\(^{[18]}\) An indirect method based on the determination of 3-MCPD content was used for determination of GE content, which involved the release of 3-MCPD and glycidol from their esters, phenylboronic acid derivatization, and quantitation using GC-MS. The internal standard, d\(_5\)-3-MCPD, and a stoichiometric conversion factor (0.67) were used for quantitation of GE. In sample preparation, two 100 mg aliquots of lipid samples were dissolved in methyl tertiary butyl ether and spiked with d\(_5\)-3-MCPD (assays A and B). Subsequently, free 3-MCPD and glycidol were released from the esters by addition of 200 μL of 25 g/L sodium methoxide in methanol. The reaction was stopped by the addition of 600 μL of acidic sodium chloride solution (200 g/L; assay A) and 600 μL of acidic sodium bromide solution (600 g/L; assay B). Quantitative analysis of the 3-MCPD derivatives was performed by a Shimadzu QP2010Plus system (Shimadzu, Tokyo, Japan) equipped with an Agilent TG-5 MS capillary column (30 m × 0.25 mm × 0.25 μm). High-purity helium was used as carrier gas with a constant flow rate of 1.18 mL/min. Each sample (1 μL) was injected in splitless mode. The GC oven temperature program was as follows: 80°C with an increase of 5°C/min to obtain 155°C, followed by increasing by 60°C/min to obtain 300°C, which was held for 5 min. The mass spectrometer was operated in selected ion monitoring mode with positive electron ionization (EI\(^+\)) at an ionization voltage of 70 eV. Temperatures of ion source and interface in mass spectrometer were 200°C and 280°C, respectively. The ion traces m/z 147 and 196 were selected as the quantitative ions for the quantitation of GE. The dwell time was set at 200 ms each. Quantification of the 3-MCPDE was conducted by multiplying the ratios of peak areas of the analyte and the internal standard d\(_5\)-3-MCPD based on corresponding ion traces with the spiking content of d\(_5\)-3-MCPD. The contents determined by assay A were the sums of 3-MCPDE and GE, and only 3-MCPDE content for assay B, so GE content could be calculated as the difference between the two determinations multiplied by a stoichiometric conversion factor, (assay A – assay B) × 0.67, and the results were expressed as glycidol equivalent units.

**Determination of the concentration of diacylglycerol in oil after deodorization**

The concentrations of diacylglycerol (DAG) in oil after deodorization were determined by HPLC-RID. The method was performed according to Han et al.\(^{[17]}\)

HPLC conditions were according to Li et al.\(^{[19]}\) Chromatographic column: Luna silica normal-phase HPLC column (2.0 mm × 250 mm, 5 μm); column temperature: 35°C; mobile phase: hexane/2-propanol (21:1, v/v) for isocratic elution; flow rate: 1.0 mL/min; injection volume: 10 μL.

Peaks in HPLC were evaluated by comparison of their retention times with those of known standards. Peak areas were calculated using integration software. The calibration curves of triacylglycerol (TAG), free fatty acid (FFA), diacylglycerol (DAG), and monoacylglycerol (MAG) were established to quantify them in oil samples by comparison of peak areas. Their concentrations in oil samples could be calculated with the reference standards.
Data analysis

Origin and SPSS were used for mapping and data processing. Experiments were carried out in triplicate independently. Data were expressed as the mean ± standard deviation. Statistical significance was measured by Duncan’s multiple range tests (P < .05).

Results and discussion

Table 1 shows the fatty acid compositions of SO, CO, PO and RO before deodorization. In Table 1, N\textsubscript{C=C} can be used as a general evaluation of unsaturated double bonds in oil. N\textsubscript{C=C} was proportional to the degree of unsaturated fatty acids in oil\textsuperscript{17} and was related to the formation of GE. We have proposed the influence mechanism of lipid oxidation on the formation of GE in the deodorization process, as shown in Figure 1. Unsaturated fatty acid (C18:3, C18:2, and C18:1) oxidation could produce free radicals (alkyl radical R· and peroxyl radical ROO·), which participated in the formation of cyclic acyloxonium free radical intermediates that finally formed GE.\textsuperscript{11} Therefore, the higher the N\textsubscript{C=C} of the oil, the more the unsaturated fatty acid in oil could promote the formation of GE.

Figure 2 shows the inhibitory effect of SO on the formation of GE in oil. Calculated content was calculated according to the content of GE in pure SO, CO, PO and RO to obtain a theoretical value of the blended oil. This calculation assumed no inhibitory effect of SO on the formation of GE in the three oils. Experimental content was determined by experiment. These values considered the inhibitory effect of antioxidants in SO on the formation of GE in the three oils. Decreased content was obtained by subtracting the experimental content from the calculated content. These values reflected the true inhibitory effect of SO on the formation of GE in the three oils. The inhibition percentage was used to evaluate the inhibitory effect of SO on the formation of GE in the three oils.

The results in Figure 2 and Table 1 are compared. In Figure 2, the experimental contents of GE in SO, CO, PO and RO followed the decreasing order of RO (1395.88 μg/kg) ≥ PO (376.84 μg/kg) ≥ CO (303.24 μg/kg) ≥ SO (133.19 μg/kg). In Table 1, the N\textsubscript{C=C} of SO, CO, PO and RO were ranked in order from high to low as CO (5.16) ≥ SO (4.72) ≥ RO (4.26) ≥ PO (2.04).

In Figure 2, the experimental content of GE in SO was the lowest, which was probably because SO contained the strongest antioxidants. The result that RO (1395.88 μg/kg) > PO (376.84 μg/kg) > CO (303.24 μg/kg) was consistent with previous research. Koyama et al. found that the content of GE in RO was 2770 μg/kg, higher than the 1080 μg/kg in PO.\textsuperscript{20} Cheng et al. found that the content of GE in PO was 1750 μg/kg, higher than the 1600 μg/kg in CO.\textsuperscript{11} Becalski et al. found that the content of GE in RO was 1250 μg/kg, higher than the 159 μg/kg in CO.\textsuperscript{21} The reason for this might be attributed to the differences in the compositions of unsaturated fatty acids (as shown in Table 1) and the concentrations of DAG (as shown in Figure 3) in the three oils.

In Figure 2, the experimental content of GE in SO was the lowest among the four oils. However, in Table 1, the N\textsubscript{C=C} of SO was the second highest, not the lowest of the four oils. This indicated that although the content of unsaturated fatty acids in SO was high (linoleic acid and oleic acid accounted for 45.50% and 41.63% in Table 1) and promoted the formation of GE, the antioxidants in SO inhibited the formation of GE. SO has outstanding antioxidant activity because of its lignans and tocopherols. Lignans such as sesamin, sesamolin, and sesamol in SO are present at approximately 6.5–17.3 g/kg. α-Tocopherol, β-tocopherol and δ-tocopherol levels in SO are approximately 56.9–99.3, 0.034–0.175, and 0.44–3.05 mg/kg, respectively.\textsuperscript{15}

In Figure 2, the experimental content of GE in CO was higher than that in SO. In Table 1, the result that the N\textsubscript{C=C} of CO was higher than that of SO indicated that the content of unsaturated fatty acids in CO was higher (linoleic acid and oleic acid accounted for 58.29% and 28.26% in Table 1) than in SO, and the content of unsaturated fatty acids in CO could promote the formation of GE in CO. This was probably because the antioxidant activity in the CO was less than in SO, could not match the ability of antioxidants in SO, and could not decrease the content of GE in CO to the lowest level as in SO. SO was a cold-pressed oil, and it retained significant natural antioxidants; however, CO was a refined oil.
Table 1. The fatty acid compositions of sesame oil, corn oil, palm oil, and rice bran oil before deodorization.

| Kind of fatty acid | Palmitic acid (%) | Stearic acid (%) | Oleic acid (%) | Linoleic acid (%) | Linolenic acid (%) | $N_{c=c}$ |
|-------------------|-------------------|------------------|----------------|-------------------|-------------------|-----------|
| SO                | 9.09 ± 0.07       | 3.78 ± 0.02      | 41.63 ± 0.27   | 45.50 ± 0.45      | -                 | 4.72      |
| CO                | 12.65 ± 0.08      | 0.80 ± 0.01      | 28.26 ± 0.15   | 58.29 ± 0.48      | -                 | 5.16      |
| PO                | 46.77 ± 0.45      | 3.21 ± 0.03      | 40.82 ± 0.34   | 8.37 ± 0.07       | -                 | 2.04      |
| RO                | 17.70 ± 0.09      | 0.78 ± 0.01      | 43.78 ± 0.36   | 37.09 ± 0.19      | 0.64 ± 0.02       | 4.26      |

SO: sesame oil; CO: corn oil; PO: palm oil; RO: rice bran oil; $N_{c=c}$: the number of moles of carbon–carbon unsaturated double bond in oil per kilogram.
that had already lost many of its natural antioxidants, such as tocopherol.\textsuperscript{[22]} Pulgarin et al. determined the antioxidant activity of SO and CO with the DPPH method. The results showed that the IC\textsubscript{50} of SO was 11.7 mg/mL, which was lower than 34.3 mg/mL of CO. This indicated that the antioxidant activity of SO was higher than that of CO. The SO contains phenols and tocopherols, including lipid-soluble lignans (sesamolinol, sesaminol, sesamin, and sesamolin) with strong antioxidant effects in foods.\textsuperscript{[23]}

In Figure 2, the experimental content of GE in PO was higher than in CO. In Table 1, the result that the N\textsubscript{C=C} of PO was the lowest of the four oils indicated that PO has the strongest oxidation stability for its high content of unsaturated fatty acids (palmitic acid accounted for 46.77\% in Table 1). The high content of GE in PO was probably attributed to the loss of antioxidants in PO. Abdullah et al. found that the amount of total phenolic content in palm oil was reduced along the various stages of the refining steps, probably due to losses through absorption by bleaching earth, volatilization and degradation during the refining process.\textsuperscript{[24]} Khrapova et al. determined the antioxidant activity of CO and PO with chemiluminescent method. The results showed that the concentration of natural antioxidants of CO was 41.6 mg/mL, which was higher than 25.6 mg/mL of PO. This indicated that the antioxidant activity of CO was higher than of PO.\textsuperscript{[25]}

Figure 1. Free radical mediated mechanism for lipid oxidation promoting the formation of glycidyl ester from diacylglycerol.
In Figure 2, the experimental content of GE in RO was higher than in PO. In Table 1, the result that the $N_{C=C}$ of RO was higher than that of PO indicated that the content of unsaturated fatty acids in RO was higher (linolenic acid, linoleic acid, and oleic acid accounted for 0.64%, 37.09%, and 43.78%,
respectively, in Table 1) than in PO (linolenic acid, linoleic acid, and oleic acid accounted for 0%, 40.82%, and 8.37%, respectively, in Table 1). Richaud et al. proved that the likelihoods of unsaturated fatty acids to be oxidized followed the order from strong to weak of C18:3 > C18:2 > C18:1.\textsuperscript{[26]} The high contents of C18:3-GE and C18:2-GE in RO were prone to oxidation and promoted the formation of GE, which led to the result that the experimental content of GE was higher than that of PO. Bhatnagar et al. determined the antioxidant activity of (50% coconut oil + 50% PO) and (50% coconut oil + 50% RO) with DPPH method. The results showed that their DPPH scavenging activity were 27.1% and 28.0%, respectively. This indicated that the antioxidant activity difference of PO and CO was not significant.\textsuperscript{[27]}

All of the discussion above suggested that the content of GE in oil could be affected by at least two factors: (i) Unsaturated fatty acids could promote the formation of GE in oil. (ii) Antioxidants in oil could inhibit the formation of GE by scavenging free radicals. The inhibitory effect of SO on the formation of GE will be discussed below.

In Figure 2, with the addition of SO increasing from 10% to 50%, the experiment content of GE decreased from 274.76 to 189.35 μg/kg in CO, decreased from 291.30 to 192.41 μg/kg in PO, and decreased from 951.14 to 557.69 μg/kg in RO. This suggested that SO could decrease the contents of GE in CO, PO and RO (P < .05). However, as the experimental content of GE in SO was lower than that in CO, RO and PO, it was possible that the decreased content of GE in the three oils was due to the dilution effect of SO.

With the addition of SO increasing from 10% to 50%, the contents of GE increased from 13.01 to 57.2 μg/kg in CO, increased from 63.39 to 103.21 μg/kg in PO, and increased from 329.94 to 417.29 μg/kg in RO. The decreased contents increased with the rising percentage of SO (P < .05). This suggested that SO could inhibit the formation of GE in CO, PO and RO.

In Figure 2, with the addition of SO increasing from 10% to 50%, the inhibition percentage increased from 4.29% to 18.86% in CO, increased from 16.82% to 27.39% in PO, and increased from 23.64% to 29.89% in RO. The inhibition percentage increased along with the increasing percentage of SO (P < .05). This suggested that SO could inhibit the formation of GE in CO, PO and RO. The inhibition percentages of SO on GE in the three oils followed the decreasing order of RO > PO > CO. This was probably because the highest content of GE in RO among the three oils could maximize the reaction between GE and antioxidants in SO, leading to the highest inhibition percentage of RO on GE.

To evaluate the inhibitory effect of SO, TBHQ was used as a blank. The usage limit of TBHQ was set at 0.02%, because the maximum content of TBHQ allowed in oil products is 0.02%, with an acceptable
daily intake of 0–0.7 mg/kg body weight in North America.\textsuperscript{[13]} In Figure 2, the decreased contents of GE in CO, PO and RO with 0.02% TBHQ added were 50.05, 107.9, and 455.89 μg/kg, respectively. These values were close to those with 50% SO added (57.02, 103.21, and 417.29 μg/kg). The inhibition percentages of TBHQ added at 0.02% on GE in CO, PO and RO were 16.50%, 28.63%, and 32.66%, respectively. These values were close to those with 50% SO added (18.86, 27.39, and 29.89 μg/kg). This indicated that when SO was added at 50%, its inhibitory effect was equivalent to that of TBHQ added at 0.02%.

Figure 3 shows the inhibitory effect of SO on the formation of diacylglycerol in oil. We have proven that both DAG and MAG could significantly increase the formation GE in oil, because they are important precursors for the formation of GE.\textsuperscript{[17]} However, MAG was not detected in this research. Therefore, the concentration of DAG in Figure 3 was closely related to the content of GE in Figure 2.

In Figure 3, the concentration of DAG was calculated according to the concentrations of DAG in pure SO, CO, PO and RO to obtain a theoretical value of the blended oil. This calculation assumed no inhibitory effect of SO on the formation of DAG in the three oils. The experimental concentration of DAG was measured by experiment. This value considered the inhibitory effect of antioxidants in SO on the formation of DAG in the three oils. Decreased concentration was obtained by subtracting the experimental concentration from the calculated concentration. This value reflected the true inhibitory effect of SO on the formation of DAG in the three oils. Decreased concentration was obtained by subtracting the experimental concentration from the calculated concentration. This value reflected the actual inhibitory effect of SO on the formation of DAG in the three oils. The inhibition percentage was used to evaluate the inhibitory effect of SO on the formation of DAG in the three oils.

In Figure 3, the experimental concentrations of DAG in SO, CO, PO, and RO followed the decreasing order of RO (11.64%) > PO (7.56%) > CO (4.11%) > SO (2.59%). This explained the reason for the result in Figure 2 that the experimental contents of GE in SO, CO, PO, and RO ranged from high to low as follows: RO (1395.88 μg/kg) > PO (376.84 μg/kg) > CO (303.24 μg/kg) > SO (133.19 μg/kg). Because DAG is an important precursor for the formation of GE, the higher the concentration of DAG in oil, the higher the content of GE.

All the discussion above suggested that the content of GE in oil could be affected by a third factor: DAG, as a precursor, could lead to the formation of GE. The inhibitory effect of SO on the formation of DAG will be discussed below.

In Figure 3, with the addition of SO increasing from 10% to 50%, the experiment concentration of DAG decreased from 3.94% to 3.39% in CO, decreased from 7.05% to 5.29% in PO, and decreased from 10.34% to 7.21% in RO. This suggested that SO could decrease the contents of DAG in CO, PO, and RO (P < .05). However, as the experimental concentration of DAG in SO was lower than that in CO, RO and PO, it was possible that the decreased contents of DAG in the three oils resulted from the dilution effect of SO.

With the addition of SO increasing from 10% to 50%, the decreased concentration of DAG increased from 0.03% to 0.21% in CO, the decreased concentration of DAG increased from 0.06% to 0.61% in PO, and the decreased concentration of DAG increased from 0.48% to 1.41% in RO. The decreased concentrations increased with the percentage of SO increasing (P < .05). This suggested that SO could inhibit the formation of DAG in CO, PO, and RO.

In Figure 3, with the addition of SO increasing from 10% to 50%, the inhibition percentage increased from 0.77% to 5.19% in CO, increased from 0.79% to 8.11% in PO, and increased from 4.12% to 12.14% in RO. The inhibition percentages increased with the percentage of SO increasing (P < .05). This suggested that SO could inhibit the formation of DAG in CO, PO, and RO. Pereira de Abreu et al. found that natural antioxidants in barley husks could slow the progress of lipid hydrolysis in cod liver oil.\textsuperscript{[22]} Thus, it was likely that antioxidants in SO inhibited the hydrolysis of triacylglycerol to diacylglycerol in CO, PO, and RO.
Conclusion

Conclusions could be drawn from the previous analysis in Figures 1–4 and Table 1 as follows. The results showed that the contents of GE in SO, CO, PO and RO followed the decreasing order of RO (1395.88 μg/kg) > PO (376.84 μg/kg) > CO (303.24 μg/kg) > SO (133.19 μg/kg). The N<sub>C=C</sub> of SO, CO, PO, and RO were in order from high to low as follows: CO (5.16) > SO (4.72) > RO (4.26) > PO (2.04). The concentrations of DAG in SO, CO, PO and RO from high to low were: RO (11.64%) > PO (7.56%) > CO (4.11%) > SO (2.59%). The content of GE in oil could be affected by at least three factors: (i) unsaturated fatty acids could promote the formation of GE in oil; (ii) antioxidants in oil could inhibit the formation of GE by scavenging free radicals; and (iii) DAG, as a precursor, could lead to the formation of GE. With the addition of SO increasing from 10% to 50%, the inhibition percentage of SO on the formation of GE increased from 4.29% to 18.86% in CO, increased from 16.82% to 27.39% in PO, and increased from 23.64% to 29.89% in RO. This suggested that SO could inhibit the contents of GE in CO, PO and RO (P < .05). The inhibition percentages of 0.02% added TBHQ on GE in CO, PO and RO were 16.50%, 28.63%, and 32.66%, respectively. This indicated that when SO was added at 50%, its inhibitory effect was equivalent to that of TBHQ added at 0.02%.

Funding

This work was financed by the National Key Research and Development Program of China (Project Nos. 2016YFD0400401-5, 2017YFC1600405-2), the National Natural Science Fund of China (Project Nos. 31401603, 31771895, 31471677), the Key Research and Development Program of Guangdong Province (Project No. 2019B020212001), Ministry of Education Engineering Research Center of Starch & Protein Processing, Guangdong Province Laboratory for Green Processing of Natural Products and Product Safety (KL-2018-11), 111 Project (B17018). This article does not contain any studies with human or animal subjects.

References

[1] Stauff, A.; Schneider, E.; Heckel, F. 2-MCPD, 3-MCPD and Fatty Acid Esters of 2-MCPD, 3-MCPD and Glycidol in Fine Bakery Wares. Eur. Food Res. Technol. 2020, 246(10), 1945–1953. DOI: 10.1007/s00217-020-03546-4.
[2] Sadowska-Rociek, A.; The Effects of Adding “Flavour Enhancers” on Levels of Chloropropanediol Esters and Glycidyl Esters in Savoury Shortbread. Eur. Food Res. Technol. 2019, 245(2), 489–498. DOI: 10.1007/s00217-018-3180-7.
[3] Kyselka, J.; Matejkova, K.; Smidrkal, J.; Bercikova, M.; Pesek, E.; Belkova, B.; Ilko, V.; Dolezal, M.; Filip, V. Elimination of 3-MCPD Fatty Acid Esters and Glycidyl Esters during Palm Oil Hydrogenation and Wet Fractionation. Eur. Food Res. Technol. 2018, 244(11), 1887–1895. DOI: 10.1007/s00217-018-3101-9.
[4] Ben Hammouda, I.; Zribi, A.; Ben Mansour, A.; Mattheaus, B.; Bouaziz, M. Effect of Deep-frying on 3-MCPD Esters and Glycidyl Esters Contents and Quality Control of Refined Olive Pomace Oil Blended with Refined Palm Oil. Eur. Food Res. Technol. 2017, 243(7), 1219–1227. DOI: 10.1007/s00217-016-2836-4.
[5] Ozdikicieler, O.; Yemiscioglu, F.; Gumuskesen, A. S. Effects of Process Parameters on 3-MCPD and Glycidyl Ester Formation during Steam Distillation of Olive Oil and Olive Pomace Oil. Eur. Food Res. Technol. 2016, 242(5), 805–813. DOI: 10.1007/s00217-015-2587-7.
[6] Dingel, A.; Matissek, R. Esters of 3-monochloropropene-12-diol and Glycidol: No Formation by Deep Frying during Large-scale Production of Potato Crisps. Eur. Food Res. Technol. 2015, 241(5), 719–723. DOI: 10.1007/s00217-015-2491-1.
[7] Frank, N.; Dubois, M.; Scholz, G.; Seefelder, W.; Chuat, J. Y.; Schiliter, B. Application of Gastrointestinal Modelling to the Study of the Digestion and Transformation of Dietary Glycidyl Esters. Food Addict Contam A. 2013, 30(1), 69–79. DOI: 10.1080/19440049.2012.732245.
[8] Abraham, K.; Hielcher, J.; Kaufholz, T.; Mielle, H.; Lampen, A.; Monien, B. The Hemoglobin Adduct N-(2,3-dihydroxypropyl)-valine as Biomarker of Dietary Exposure to Glycidyl Esters: A Controlled Exposure Study in Humans. Arch. Toxicol. 2019, 93(2), 331–340. DOI: 10.1007/s00204-018-2373-y.
[9] Liu, R.; Guo, X.; Cheng, M.; Zheng, L.; Gong, M.; Chang, M.; Jin, Q.; Wang, X.; Chew, S.-C.; Tan, C.-P. Effects of Chemical Refinement on the Quality of Coconut Oil. J Food Sci Tech Mys. 2019, 56(6), 3109–3116. DOI: 10.1007/s13197-019-03810-w.
[10] Chew, S. C.; Tan, C. P.; Lai, O. M.; Nyam, K. L. Changes in 3-MCPD Esters, Glycidyl Esters, Bioactive Compounds and Oxidation Indexes during Kenaf Seed Oil Refining. Food Sci Biotechnol. 2018, 27(3), 905–914. DOI: 10.1007/s10068-017-0295-8.
[11] Cheng, W.; Liu, G.; Liu, X. Effects of Fe\(^{3+}\) and Antioxidants on Glycidyl Ester Formation in Plant Oil at High Temperature and Their Influencing Mechanisms. J. Agr. Food Chem. 2017, 65(20), 4167–4176. DOI: 10.1021/acs.jafc.7b00858.

[12] Gharavi, N.; Haggarty, S.; Aos, E.-K. Chemoprotective and Carcinogenic Effects of tert-butylhydroquinone and Its Metabolites. Curr. Drug Metab. 2007, 8(1), 1–7. DOI: 10.2174/138920007779315035.

[13] Taghvaei, M.; Jafari, S. M. Application and Stability of Natural Antioxidants in Edible Oils in order to Substitute Synthetic Additives. J Food Sci Tech Mys. 2015, 52(3), 1272–1282. DOI: 10.1007/s13197-013-1080-1.

[14] Wong, Y. H.; Goh, K. M.; Nyam, K. L.; Nehdi, I. A.; Shibii, H. M.; Tan, C. P. Effects of Natural and Synthetic Antioxidants on Changes in 3-MCPD Esters and Glycidyl Ester in Palm Olein during Deep-fat Frying. Food Control. 2019, 96, 488–493. DOI: 10.1016/j.foodcont.2018.10.006.

[15] Wan, Y.; Li, H.; Fu, G.; Chen, X.; Chen, F.; Xie, M. The Relationship of Antioxidant Components and Antioxidant Activity of Sesame Seed Oil. J. Sci. Food Agr. 2015, 95(13), 2571–2578. DOI: 10.1002/jsfa.7035.

[16] Cheng, W.; Liu, G.; Wang, X.; Liu, X.; Liu, B. Formation of Benzo(a)pyrene in Sesame Seeds during the Roasting Process for Production of Sesame Seed Oil. J. Am. Oil Chem. Soc. 2015, 92(11–12), 1725–1733. DOI: 10.1007/s11746-015-2734-0.

[17] Han, L.; Lin, Q.; Liu, G.; Han, D.; Niu, L.; Su, D. Lipids Promote Glycated Phospholipid Formation by Inducing Hydroxy Radical in a Maillard Reaction Model System. J. Agr. Food Chem. 2019, 67(28), 7961–7967. DOI: 10.1021/acs.jafc.9b02771.

[18] Cheng, W.; Liu, G.; Liu, X. Formation of Glycidyl Fatty Acid Esters Both in Real Edible Oils during Laboratory-scale Refining and in Chemical Model during High Temperature Exposure. J. Agr. Food Chem. 2016, 64(29), 5919–5927. DOI: 10.1021/acs.jafc.6b01520.

[19] Li, D.; Wang, W.; Durrani, R.; Li, X.; Yang, B.; Wang, Y. Simplified Enzymatic Upgrading of High-acid Rice Bran Oil Using Ethanol as a Novel Acyl Acceptors. J. Agr. Food Chem. 2016, 64(35), 6730–6737. DOI: 10.1021/acs.jafc.6b02518.

[20] Koyama, K.; Miyazaki, K.; Abe, K.; Egawa, Y.; Kido, H.; Kitta, T.; Miyashita, T.; Nezu, T.; Nohara, H.; Sano, T.; et al. Collaborative Study of an Indirect Enzymatic Method for the Simultaneous Analysis of 3-MCPD, 2-MCPD, and Glycidyl Esters in Edible Oils. J. Oleo Sci. 2016, 65(7), 557–568. DOI: 10.5650/jos.ess16021.

[21] Becalski, A.; Feng, S.; Lau, B. P. Y.; Zhao, T. A Pilot Survey of 2-and 3-monochloropropanediol and Glycidol Fatty Acid Esters in Foods on the Canadian Market 2011–2013. J. Food Compos. Anal. 2015, 37, 58–66. DOI: 10.1016/j.jfca.2014.09.002.

[22] De Abreu, D. A. P.; Rodriguez, K. V.; Cruz Freire, J. M., Pereira de Abreu DA, Villalba Rodriguez K, Cruz Freire JM. Effectiveness of Antioxidants on Lipid Oxidation and Lipid Hydrolysis of Cod Liver Oil. Eur. J. Lipid Sci. Tech. 2011, 113(11), 1395–1401. DOI: 10.1002/ejlt.201100189.

[23] Pulgarin, J. A. M.; Bermejo, L. F. G.; Duran, A. C. Evaluation of the Antioxidant Activity of Vegetable Oils Based on Luminol Chemiluminescence in a Microemulsion. Eur. J. Lipid Sci. Tech. 2010, 112(12), 1294–1301. DOI: 10.1002/ejlt.201000364.

[24] Abdullah, F.; Ismail, R.; Ghazali, R.; Idris, Z. Total Phenolic Contents and Antioxidant Activity of Palm Oils and Palm Kernel Oils at Various Refining Process. J. Oil Palm Res. 2018, 30, 682–692.

[25] Khrapova, N. G.; Skibida, I. P.; Misin, V. M. The Kinetic Characteristics of Natural Antioxidants in Vegetable Oils. Russ. J. Phys. Chem. B. 2010, 112(12), 1294–1301.

[26] Richaud, E.; Audouin, L.; Fayolle, B.; Verdu, J.; Matisova-Rychla, L.; Rychly, J. Rate Constants of Oxidation of Unsaturated Fatty Esters Studied by Chemiluminescence. Chem. Phys. Lipids. 2012, 165(7), 753–759. DOI: 10.1016/j.chemphyslip.2012.09.002.

[27] Bhatnagar, A. S.; Kumar, P. K. P.; Hemavathy, J.; Krishna, A. G. G. Fatty Acid Composition, Oxidative Stability, and Radical Scavenging Activity of Vegetable Oil Blends with Coconut Oil. J. Am. Oil Chem. Soc. 2009, 86(10), 991–999. DOI: 10.1007/s11746-009-1435-y.