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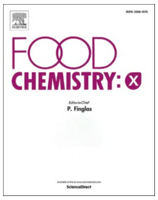

Antioxidant mechanism of ferulic acid and γ-oryzanol in emulsion and emulgel based on black seed oil

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ABSTRACT

The aim of this research was to compare the antioxidant mechanism of ferulic acid and γ-oryzanol in emulsion with that of emulgel. In the initiation phase, ferulic acid at 2.32 mM concentration exhibited the best efficiency in emulgel samples, while in emulsion samples, γ-oryzanol at 2.32 mM concentration exhibited the best efficiency. γ-Oryzanol was more effective than ferulic acid in emulsion and emulgel samples in the propagation phase. The γ-oryzanol and ferulic acid participated in side reactions of initiation chain in addition to participating in the major termination reaction. In both of the emulgel and emulsion samples, ferulic acid showed higher consumption rate constant and extent of participation in side reactions of initiation chain than that of γ-oryzanol. In conclusion, ferulic acid showed higher efficiency in emulgel in the initiation phase, while γ-oryzanol showed higher efficiency in emulsion and emulgel in the propagation phase.

1. Introduction

Black seed (*Nigella sativa*) oil contains several phytochemicals ranging from polyphenols to alkaloids. This oil has beneficial effects such as anti-cancer, anti-diabetic, (Mazaheri, Torbati, Azadmard-Damirchi, & Savage, 2019) antimicrobial, and anti-hypertensive properties (Alkhatib, Assadpour, Sabere, Mohamed, & Jafari, 2022). Thymoquinone is the main biologically active molecule in black seed oil (Kooti, Hasanzadeh-Noohi, Sharafi-Ahvazi, Asadi-Samani, & Ashtary-Larky, 2016). In black seed oil, 74.8–77.5 % of total fatty acids are unsaturated. The most abundant unsaturated fatty acids in black seed oil are linoleic acid followed by oleic acid, and α-linolenic acid (Mazaheri et al., 2019). Although polyunsaturated fatty acids can reduce cardiovascular disease, but they are highly susceptible to peroxidation. However, low water-solubility of black seed oil limits its application in food products (Nor Hayati, Hui, Ishak, Mohd Yusof, & Muhamad Hanidun, 2020).

Recently, oil-in-water emulsions have been applied as efficient vehicles for delivering black seed oil into food products (Nor Hayati et al., 2020). The existence of significant percentage of polyunsaturated fatty acids in black seed oil makes it susceptible to peroxidation reaction especially in the emulsified form (Rahim et al., 2022). Peroxidation process in oil-in-water emulsions is more complicated than in bulk oils. In general, the peroxidation rate in oil-in-water emulsion is higher than that of bulk oil. This is due to the existence of an interfacial layer between the water phase and the lipid phase. This interfacial layer can enhance the reaction rate between fatty acids in the lipid phase and prooxidant compounds in the continuous phase. Addition of efficient antioxidants that can concentrate at the interfacial layer can inhibit peroxidation rate (Keramat, Ehsandoost, & Golmakani, 2023; Villeneuve et al., 2023).

Ferulic acid (FA) is a phenolic compound that exists in plant tissues. FA possess several biological effects such as anti-hypertensive, anti-diabetic, blood sugar reduction, anticancer, anti-inflammatory, cardioprotective, and radical scavenging effects (Fang et al., 2024). The phenolic nucleus and carboxylic side chain of FA creates a resonance stabilized phenoxy radical. The radical scavenging effect of FA is related to this resonance stabilized phenoxy radical (Srinivasan, Sudheer, & Menon, 2007). γ-Oryzanol (GO) is the ester of FA with phytosterols. This compound mainly exists in rice bran oil and possess several biological activities such as free radical scavenging, neuromodulation, and anti-ulcer effects. Both phenolic hydroxyl group and sterol (triterpene) moiety of GO can exert antioxidant activity (Lerma-García, Herrero-Martínez, Simó-Alfonso, Mendonça, & Ramis-Ramos, 2009). GO has

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lower ratio of hydrophilic/hydrophobic moieties than that of FA, resulting in higher solubility of GO in lipid substrate (Nenadis, Zhang, & Tsimidou, 2003).

Another novel method for inhibiting the peroxidation rate of unsaturated lipids is embedding emulsion droplets in emulgel structures (Keramat & Golmakani, 2024b; Keramat, Golmakani, Niakousari, & Toorani, 2023). Emulgel is produced through immobilizing emulsion droplets in a gel network (Cui, Guo, & Meng, 2023). Proteins (e.g., whey protein, gelatin, casein, and soy protein), polysaccharides (e.g., xanthan gum, starch, alginate, and pectin), or combinations of protein and polysaccharide can be applied as crosslinking biopolymer for emulgel production (Abdullah Liu, Javed, & Xiao, 2022). It has been reported that emulgels containing linseed oil and sunflower oil exhibited higher resistance to peroxidation than those emulsions containing these oils. This can be attributed to the limited movement of pro-oxidants in the aqueous phase toward unsaturated lipids in the oil phase, resulting in lower peroxidation rate of emulgel (Keramat & Golmakani, 2024a; Keramat, Niakousari, & Golmakani, 2023).

In dispersed systems, the hydrophilic-lipophilic balance value of antioxidant (which can affect the location of antioxidants) (Noon, Mills, & Norton, 2020), physicochemical characteristic of interfacial layer (Leiva-Vega et al., 2020), mass transport phenomena and the ability of antioxidant molecules to transfer toward the interfacial layer (Keramat & Golmakani, 2024a; Keramat, Golmakani, et al., 2023; Laguerre, Bily, Roller, & Birtić, 2017), droplet size (Erdmann et al., 2017), oil percentage (Costa et al., 2020) and pH (Zhou & Elias, 2013) can impact the antioxidant activity. However, each antioxidant compound shows different behavior based on its molecular structure and the surrounding medium (Villeneuve et al., 2023).

The antioxidant activity of FA and GO in oil-in-water emulsion have been investigated in some studies (Cantele et al., 2023; Liu, Xu, Chang, Liu, & Wang, 2021). However, there is few information regarding their antioxidant mechanism of action and different antioxidant pathways that they participate in oil-in-water emulsion. Besides, the antioxidant capacity of FA and GO in emulgel has not been studied. Several research have studied the application of emulgels as fat replacer in food products (Chaijan, Cheong, & Panpipat, 2021; Zare et al., 2024). However, the lipid oxidation and the mechanism of action of antioxidants in emulgel has gained less attention. The physical and chemical characteristics of oil dispersion and the interaction of antioxidants with other components in oil dispersion can affect the antioxidant mechanism in oil dispersion (Keramat & Golmakani, 2024a; Qiu, Jacobsen, Villeneuve, Durand, & Sørensen, 2017). Therefore, in this study, the antioxidant mechanism of FA and GO and different antioxidant pathways in which FA and GO take part in emulsion was compared to that of emulgel containing black seed oil for the first time. The antioxidant functions of FA and GO in emulsion and emulgel were investigated through a sigmoidal kinetic model.

2. Materials and methods

2.1. Materials

Antioxidant free black seed oil was supplied from a local market. Chloroform and isooctane were supplied from Merck Company (Darmstadt, Germany). FA, ferrous sulfate, ammonium thiocyanate, GO, 2,2 diphenyl-1-picrylhydrazyl radical (DPPH$^\bullet$), and barium chloride were supplied from Sigma-Aldrich Company (St. Louis, MO). Methanol, isopropanol, and ethanol were supplied from PARS Chemie Company (Tehran, Iran).

2.2. Radical scavenging effect of FA and GO

FA and GO radical scavenging effects were measured using DPPH assay. FA and GO were solubilized in ethanol at various concentrations (5.15–515.00 μM) to produce stock solution. DPPH was dissolved in ethanol at 135 μM concentration. Then, DPPH solution (1 mL) was separately mixed with FA and GO stock solutions (1 mL). To obtain the kinetic curves of [DPPH$^\bullet$] reduction, the absorbance values of FA and GO samples were determined at 517 nm during 3 h storage at ambient temperature. To determine the concentration of DPPH$^\bullet$ in the reaction mixture, a standard curve of DPPH$^\bullet$ absorbance in the concentration range of 0–135 μM was plotted (Shojaee, Moeenfard, & Farhoosh, 2022).

When FA or GO exists in high concentrations, DPPH$^\bullet$ reduction during storage is calculated using a pseudo-first-order kinetic model (Eq. (1)).

$$[\text{DPPH}^\bullet] = [\text{DPPH}_0].e^{-k1t} \tag{1}$$

where t is time (min), s_1 (min^{-1}) is the pseudo-first-order rate constant, and $[\text{DPPH}^\bullet]_0$ is initial concentration of DPPH$^\bullet$ (Thavasi, Bettens, & Leong, 2009).

The second order (bimolecular) rate constant (s_2, M^{-1} min^{-1}) is calculated using the slope of s_1 curve against FA or GO concentration (Mishra, Ojha, & Chaudhury, 2012).

The IR values (initial reaction rate, μmol DPPH$^\bullet$ min^{-1}) at the same FA or GO and DPPH$^\bullet$ concentration (135 μM) were determined using Eq. (2) at $t = 0.5$ min (Shojaee et al., 2022).

$$\text{IR}_i = -[\text{DPPH}^\bullet]_0.k_1.e^{-k1t} \tag{2}$$

The n value (number of DPPH$^\bullet$ mole inhibited by one FA or GO mole) was determined by Eq. (3).

$$n = \frac{[\text{DPPH}^\bullet]_0}{2 \times \text{IC}_{50}} \tag{3}$$

The IC$_{50}$ value (FA or GO concentration needed for reduction of half of the initial concentration of DPPH$^\bullet$) was measured from the regression analysis of [DPPH$^\bullet$] plot against FA or GO concentration (Shojaee et al., 2022).

2.3. Partition coefficient of FA and GO

The partition coefficients (log P values) of FA and GO were measured using Molinspiration software (Molinspiration Cheminformatics, Bratislava, Slovak Republic). The log P value is an indicator of antioxidant hydrophobicity (Keramat, Golmakani, Durand, Villeneuve, & Hosseini, 2021). Method for determining the log P value is based on group contributions. The calculated log P values have been fitted with experimental log P values for more than 12,000 molecules. By using this method, hydrophobicity values for 35 small simple “basic” fragments and 185 larger fragments have been obtained (Lalitha & Sivakamasundari, 2010).

2.4. Fatty acid composition of black seed oil

Fatty acid profile of black seed oil was measured by a gas chromatography apparatus (SP-3420 A, Beijing Beifen-Ruili analytical instrument, China) using the method explained by Golmakani, Keramat, Niakousari, and Khosravi (2018). A flame ionization detector, a split/splitless injector, and a BPX70 capillary column (Bis-cyanopropylsiloxane-silphenylene; 30 m length, 0.25 mm internal diameter, and 0.25 μm film thickness) were applied. Palmitic acid (16.34 %), oleic acid (16.75 %), and linoleic acid (65.99 %) were the major fatty acids present in blackseed oil (Fig. 1). Palmitic acid, oleic acid, and linoleic acid were appeared at the retention times of 27, 31, and 32 min, respectively.

2.5. Preparation of emulsion and emulgel based on black seed oil

FA and GO were separately added to black seed oil at 1.03, 2.32, 3.61, 4.89, 6.18 mM. Acetone was used as a solvent for incorporation of FA and GO into black seed oil. To prepare pre-emulsion samples, at first, Tween 80 (0.88 g) was dispersed drop by drop into potassium phosphate buffer (25 g, 0.04 mmol/L, pH 7) during 15 min, while mixing with the

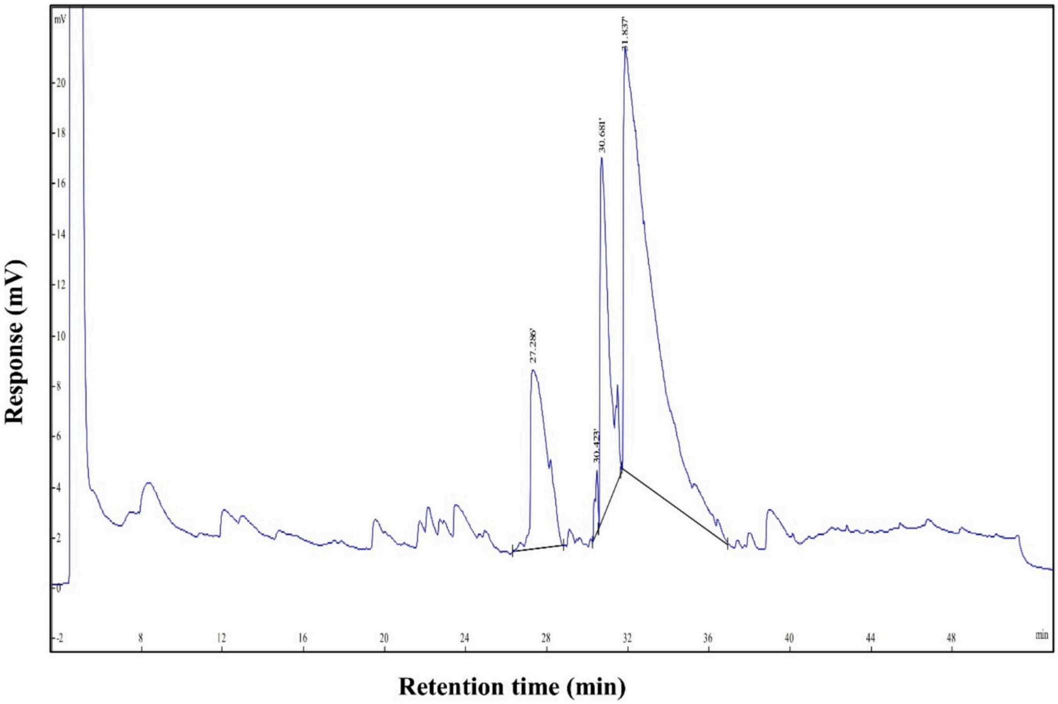

Fig. 1. Gas chromatography/flame ionization detector chromatogram of blackseed oil.

buffer in a magnetic stirrer at 700 rpm and 25 °C. Then, black seed oil (2.5 g) containing FA and GO added to the continuous phase at 300 μL min^{-1} rate, while mixing with the buffer in a magnetic stirrer at 700 rpm and 25 °C. An ULTRA-TURRAX homogenizer (T-25, IKA, Germany) was used for preparation of coarse emulsion. The stirring speed was 13,500 rpm, the homogenization time was 5 min at 25 °C. Prepared pre-emulsions were treated by ultrasonic homogenizer (JY96-IIN, 150 W, SCIENTZ, Zhejiang, China) to produce stable and fine emulsions. The probe was placed about 1 cm below the emulsion surface, while the volume of the sample was 20 cm^3. The optimal time of ultrasound emulsification was also 5 min. To obtain optimal ultrasound conditions, pulsed treatment was applied: 7:3 s (On:Off). During pulsation temperature was maintained below 25 °C using ice-bath (Nejadmansouri, Hosseini, Niakosari, Yousefi, & Golmakani, 2016).

The produced black seed oil-in-water emulsion was used for emulgel production. The emulsion temperature was enhanced to 80 °C during 15 min. After that, kappa-carrageenan was incorporated into the emulsion at 3 % (*w*/w). The mixture of emulsion and kappa-carrageenan were mixed on a magnetic stirrer at 80 °C for 20 min. Finally, the produced emulgel was kept at refrigerator for one day (Kamlow, Spyropoulos, & Mills, 2021).

2.6. *Rheological characteristic of emulgel based on black seed oil*

The rheological characteristics of emulgel containing black seed oil was investigated using a controlled stress/strain rheometer (MCR 302, Anton Paar, Graz, Austria). A parallel plate geometry (gap size and diameter of 1 mm and 40 mm, respectively) was applied for determining rheological behavior. The amplitude sweep test at 6.28 rad/s frequency and in the shear stress range of 0.001–25 Pa were applied for determination of the linear viscoelastic region. The frequency sweep tests were done at 0.01 % strain. Both of the amplitude sweep test and frequency sweep test were done at 25 °C.

2.7. *Droplet size of emulgel and emulsion based on blackseed oil*

The droplet size of emulgel and emulsion based on blackseed oil was determined via a dynamic light scattering instrument (SZ-100 nanopartica series, Horiba ltd., Kyoto, Japan). Emulgel and emulsion samples were diluted 1:100 with potassium phosphate buffer 0.04 mmol/L, pH 7). The light scattering angle was 173°. The droplet size of emulgel and emulsion samples were 106.93 ± 1.50 and 120.23 ± 4.54 nm, respectively.

2.8. *Kinetic study*

The amounts of hydroperoxides of emulgel and emulsion samples were determined during storage at 45 °C. The amounts of hydroperoxides were measured by determining the absorbance at 510 nm according to the International Dairy Federation assay (Shantha & Decker, 1994). For each sample, plots of hydroperoxide concentrations (LOOH) were obtained against time (t). The s_i value (pseudo-zero order rate constant) was measured by Eq. (4).

$$\frac{d[\text{LOOH}]}{dt} = s_i \tag{4}$$

The LOOH formation plots in the initiation and propagation phases were described using a sigmoidal model (Eq. (5)).

$$[\text{LOOH}] = \frac{s_f}{exp\left[s_f(\text{C-t})\right] + s_d} \tag{5}$$

where s_d (mM^{-1} h^{-1}) and s_f (h^{-1}) are pseudo-second order rate constant and pseudo-first order rate constant of LOOH production in the propagation phase. In addition, C (mM^{-1}) is integration constant.

The IP value (induction period, h) was measured using Eq. (6).

$$\text{IP} = \frac{s_f\left(2\text{-}s_f\text{C} + \ln s_d\right)\text{-}4[\text{ROOH}]_0 s_d}{4s_i s_d\text{-}s^2{}_c} \tag{6}$$

The AF value (FA or GO effectiveness for peroxyl radical inhibition in the initiation phase) was measured using Eq. (7).

$$\text{AF} = \frac{\text{IP}_\text{A}}{\text{IP}_\text{O}} \tag{7}$$

where IP_A and IP_O are the IP values of samples treated with FA or GO and IP value of the control sample, respectively.

The oxidation rate ratio (OR) was measured by Eq. (8).

$$\mathrm{OR} = \frac{\mathrm{s}_{\mathrm{i,A}}}{\mathrm{m}_{\mathrm{i,O}}} \tag{8}$$

where $\mathrm{s}_{\mathrm{i,\,A}}$ and $\mathrm{m}_{\mathrm{i,\,O}}$ are the s_{i} values of the samples containing FA or GO and the control sample, respectively.

The A value (activity) was measured using Eq. (9).

$$\mathrm{A} = \frac{\mathrm{AF}}{\mathrm{OR}} \tag{9}$$

The r_{AC} value (mean rate of FA or GO consumption, mM h^{-1}) was measured by Eq. (10).

$$\mathrm{r}_{\mathrm{AC}} = \frac{[\mathrm{AC}]_0}{\mathrm{IP}_{\mathrm{A}}} \tag{10}$$

The TP value (turning point, h), which is the time when the LOOH production rate reaches the maximum value was measured using Eq. (11).

$$\mathrm{TP} = \frac{\mathrm{s}_{\mathrm{f}}\mathrm{C}\text{-}\ln \mathrm{s}_{\mathrm{d}}}{\mathrm{s}_{\mathrm{f}}} \tag{11}$$

The $\mathrm{LOOH}_{\mathrm{m}}$ (the highest LOOH concentration (mM)) was measured using Eq. (12).

$$[\mathrm{LOOH}]_{\mathrm{m}} = lim t \to \infty \left\{ \frac{s_{\mathrm{f}}}{exp\left[s_{\mathrm{f}}(\mathrm{C}-\mathrm{t})\right] + s_{\mathrm{d}}} \right\} \frac{\mathrm{s}_{\mathrm{f}}}{\mathrm{s}_{\mathrm{d}}} \tag{12}$$

The M_{R} value (the highest LOOH formation rate during the propagation phase (mM h^{-1}) was measured using Eq. (13).

$$M_R = \left(\frac{\mathrm{d}[\mathrm{LOOH}]}{\mathrm{dt}}\right)_{\mathrm{max}} = \frac{s^2{}_{\mathrm{f}}}{4s_{\mathrm{d}}} \tag{13}$$

The P_{O} value (propagation oxidizability, h^{-1}) was measured using Eq. (14).

$$\mathrm{P}_{\mathrm{O}} = \frac{\mathrm{M}_{\mathrm{R}}}{[\mathrm{LOOH}]_{\mathrm{max}}} \tag{14}$$

The ET value (end time of the propagation phase, h) was measured using Eq. (15).

$$\mathrm{ET} = \frac{4\mathrm{s}_{\mathrm{d}}\mathrm{M}_{\mathrm{R}}\text{-}\mathrm{s}_{\mathrm{f}}\mathrm{P}_{\mathrm{o}}\left(2\text{-}\mathrm{s}_{\mathrm{f}}\mathrm{C} + \ln \mathrm{s}_{\mathrm{d}}\right)}{4\mathrm{s}_{\mathrm{d}}\mathrm{M}_{\mathrm{R}}\mathrm{P}_{\mathrm{O}}} \tag{15}$$

The PP value (propagation period, h) was measured using Eq. (16) (Farhoosh, 2021).

$$\mathrm{PP} = \mathrm{ET} - \mathrm{IP} \tag{16}$$

2.9. *Antioxidant mechanism of FA and GO in emulgel and emulsion based on black seed oil*

The antioxidant mechanism of FA and GO is the result of taking part of FA and GO molecules (A) and radicals ($\mathrm{A}^{\bullet}$) in different reactions (Fig. 2). The AF value indicates the possibility of scavenging lipid peroxyl radical ($\mathrm{LOO}^{\bullet}$) (Toorani, Golmakani, & Gahruie, 2020).

The possibility of FA or GO radical for participating in the reactions −7, 10, and/or 14 (side reactions of the chain propagation) is determined using Eq. ($^{\bullet}$) via reaction 7, which is the major chain termination reaction. Linear relationship between AF value and AC_0 value (initial FA or GO concentration) indicates that FA or GO mainly takes part in reaction 7 (Denisov & Khudyakov, 1987).

Nonlinear relationship between AF value and AC_0 value indicates that FA or GO takes part in reactions 11 and/or 12 (side reactions of initiation chain) beside reaction 7. The possibility of FA or GO for participating in reactions 11 and 12 can be elucidated using Eq. (17).

0. $2\mathrm{LH} + \mathrm{O}_2 \rightarrow 2\mathrm{L}^{\bullet} + \mathrm{H}_2\mathrm{O}_2$

1. $\mathrm{L}^{\bullet} + \mathrm{O}_2 \rightarrow \mathrm{LOO}^{\bullet}$

2. $\mathrm{LOO}^{\bullet} + \mathrm{LH} \rightarrow \mathrm{LOOH} + \mathrm{L}^{\bullet}$

3. $\mathrm{LOOH} \rightarrow \mathrm{LO}^{\bullet} + {}^{\bullet}\mathrm{OH}$

3′. $2\mathrm{LOOH} \rightarrow \mathrm{LOO}^{\bullet} + \mathrm{LO}^{\bullet} + \mathrm{H}_2\mathrm{O}$

3″. $\mathrm{LOOH} + \mathrm{LH} \rightarrow \mathrm{LO}^{\bullet} + \mathrm{L}^{\bullet} + \mathrm{H}_2\mathrm{O}$

4. $\mathrm{L}^{\bullet} + \mathrm{L}^{\bullet} \rightarrow \mathrm{L\text{-}L}$

5. $\mathrm{L}^{\bullet} + \mathrm{LOO}^{\bullet} \rightarrow \mathrm{LOOL}$

6. $\mathrm{LOO}^{\bullet} + \mathrm{LOO}^{\bullet} \rightarrow$ Non-radical products

7. $\mathrm{LOO}^{\bullet} + \mathrm{AH} \rightarrow \mathrm{LOOH} + \mathrm{A}^{\bullet}$

-7. $\mathrm{A}^{\bullet} + \mathrm{LOOH} \rightarrow \mathrm{AH} + \mathrm{LOO}^{\bullet}$

8. $\mathrm{A}^{\bullet} + \mathrm{LOO}^{\bullet} \rightarrow \mathrm{AOOL}$

9. $\mathrm{A}^{\bullet} + \mathrm{A}^{\bullet} \rightarrow$ Products

10. $\mathrm{A}^{\bullet} + \mathrm{LH} \rightarrow \mathrm{AH} + \mathrm{L}^{\bullet}$

11. $\mathrm{LOOH} + \mathrm{AH} \rightarrow \mathrm{LO}^{\bullet} + \mathrm{A}^{\bullet} + \mathrm{H}_2\mathrm{O}$

12. $\mathrm{AH} + \mathrm{O}_2 \rightarrow \mathrm{A}^{\bullet} + \mathrm{HOO}^{\bullet}$

13. $\mathrm{A\text{-}OOL} \rightarrow \mathrm{A}^{\bullet} + \mathrm{LO}^{\bullet}$

14. $\mathrm{A}^{\bullet} + \mathrm{O}_2 \rightarrow \mathrm{AOO}^{\bullet}$

Fig. 2. Oxidation reactions in the absence (0–6) and presence (7–14) of ferulic acid or γ-oryzanol molecules (AH). LOOH, lipid hydroperoxide; LH, lipid reactant; $\mathrm{A}^{\bullet}$, ferulic acid or γ-oryzanol radicals; $\mathrm{LOO}^{\bullet}$, peroxyl radical; $\mathrm{L}^{\bullet}$, lipid radical; $\mathrm{LO}^{\bullet}$, alkoxy radical (Denisov & Khudyakov, 1987).

$$\mathrm{r}_{\mathrm{AC}} = \mathrm{R}_{\mathrm{eff}}[\mathrm{AC}]_0^{\mathrm{n}} + \frac{\mathrm{Ki}}{\mathrm{a}} \tag{17}$$

where $\mathrm{R}_{\mathrm{eff}}$ is the rate constant of FA or GO consumption in side reactions of initiation chain, n is the reaction order, a is the stoichiometric coefficient of scavenging radicals which shows the number of radicals scavenged by one FA or GO molecule, and K_{i} is the average rate in the initiation phase. A zero-order ($n = 0$) rate side reaction indicates that FA or GO do not participate in the side reactions of initiation chain. Also, first-order ($n = 1$) rate means that FA or GO take part in one of the side reactions of initiation chain. In addition, second-order ($n = 2$) rate shows that FA or GO takes part in both reactions 11 and 12 18).

$$s_{i,A} : [AC]_0^n \tag{18}$$

Linear relationship at n = (−1) means that FA or GO radicals do not take part in the chain propagation reactions. Also, no dependency (n = 0) shows that FA or GO are too active and the reaction rate of FA or GO with lipid peroxyl radical is higher than that of lipid reactant with lipid peroxyl radical. In addition, linear relationship at n = (−0.5) means that FA or GO radicals mainly take part in reaction 10. Furthermore, nonlinear relationship at n = (−0.5) and (−1) means that FA or GO radicals participate in at least two chain propagation reactions. (Toorani et al., 2020).

2.10. Statistical analysis

The ANOVA (one-way analysis of variance) was used for elucidating significant differences. Comparing the mean values was done by Duncan's multiple range test ($P < 0.05$). Regression analyses were done by Microsoft Office Excel and CurveExpert software and statistical analyses were done by SPSS software.

3. Results and discussion

3.1. Radical scavenging activity of FA and GO

Kinetic parameters of FA and GO are presented in Table 1. FA showed higher IR and s_2 values than those of GO. This can be related to the stronger solvent-based dynamism of FA to scavenge DPPH●. It is expected that polar solvents can establish better dynamic reaction environment for antioxidant compounds with higher polarity. In fact, in polar solvents, antioxidants with higher polarity can establish more effective collisions with DPPH● than those with lower polarity (Shojaee et al., 2022). In this study, ethanol that is a polar compound (log P of −0.18) was used as a solvent. Therefore, the higher reaction rate of DPPH● with FA can be related to the higher polarity of FA (log P of 1.42) than that of GO (log P of 10.12).

In addition to the reaction rate of DPPH● with FA and GO, the reaction stoichiometry is another important parameter for evaluating the radical scavenging activity. The n value, is the maximum number of DPPH● moles reduced by one mole of FA or GO. FO showed higher n value than that of GO. Also, FA showed lower IC_{50} value than that of GO (Table 1). Therefore, FA showed higher ability to scavenge DPPH● than that of GO. This can be related to the better steric accessibility of FA than that of GO for reacting with DPPH●. The steric accessibility of FA and GO hydroxyl groups to the radical site of DPPH● can significantly affect the radical scavenging activities of FA and GO. Large antioxidant molecules with higher complexity exhibits lower steric accessibility since these molecules have low ability to rotate and orient reactive groups toward the DPPH● (Xie & Schaich, 2014). GO is the ester of ferulic acid with phytosterol. Therefore, the lower radical scavenging potency of GO than FA may be due to the presence of bulky phytosterol group in GO that reduced the accessibility of phenolic hydroxyl group to the radical site of DPPH●.

Table 1
Kinetic parameters and IC_{50} values of ferulic acid and γ-oryzanol in radical scavenging capacity assay.

Kinetic parameter	Ferulic acid	γ-Oryzanol
IR_i (nmol DPPH● min^{-1}) × 10	$1.89 \pm 0.27^{a*}$	1.01 ± 0.14^{b}
$s_2 \times 10^6$ (μM^{-1} min^{-1})	20.00 ± 0.03^{a}	4.50 ± 0.71^{b}
IC_{50} (μM)	127.79 ± 2.73^{b}	182.10 ± 5.14^{a}
n	0.53 ± 0.01^{a}	0.37 ± 0.02^{b}

[*] Mean ± SD ($n = 3$). In each column, means with different superscript letters are significantly different ($P < 0.05$). IR_i: initial reaction rates at an equal (135 μM) concentration of DPPH● and ferulic acid or γ-oryzanol, s_2: second-order rate constant, IC_{50}: the antioxidant concentration required for scavenging 50 % of the initial [DPPH●], and n: the number of the mole of DPPH● reduced by one mole of antioxidant.

3.2. Rheological characteristics of emulgel based on black seed oil

The rheological characteristics of emulgel was investigated by amplitude (Fig. 3a) and frequency sweep (Fig. 3b) tests. In the amplitude sweep test, the G″ values (loss modulus) of emulgel were lower than those of G′ values (storage modulus) at shear stress lower than 10 Pa. This shows elastic behavior of emulgel. At shear stress higher than 10 Pa, the G″ value was higher than that of G′ value. Accordingly, a transition from elastic behavior to viscose behavior was observed. In the frequency sweep test, the G″ values of emulgel were lower than those of G′ values in the investigated frequency range. Accordingly, emulgel exhibited elastic characteristic rather than viscous characteristic.

3.3. Effect of FA and GO on the emulgel and emulsion oxidation kinetic in the initiation phase

The IP value of control emulgel and emulsion samples were 125.11 and 120.76 h, respectively (Table 2). In addition, the s_i values of control emulgel and emulsion samples were 0.05 and 0.02 mM h^{-1}, respectively. The higher values of IP and lower values of s_i show lower oxidation rate. Accordingly, control emulgel sample showed lower oxidation rate than that of control emulsion sample. The lower oxidation rate of emulgel sample than that of emulsion sample can be explained by mass transport phenomena. In lipid dispersion, the ability of pro-oxidant compounds to transfer toward unsaturated lipid can significantly affect

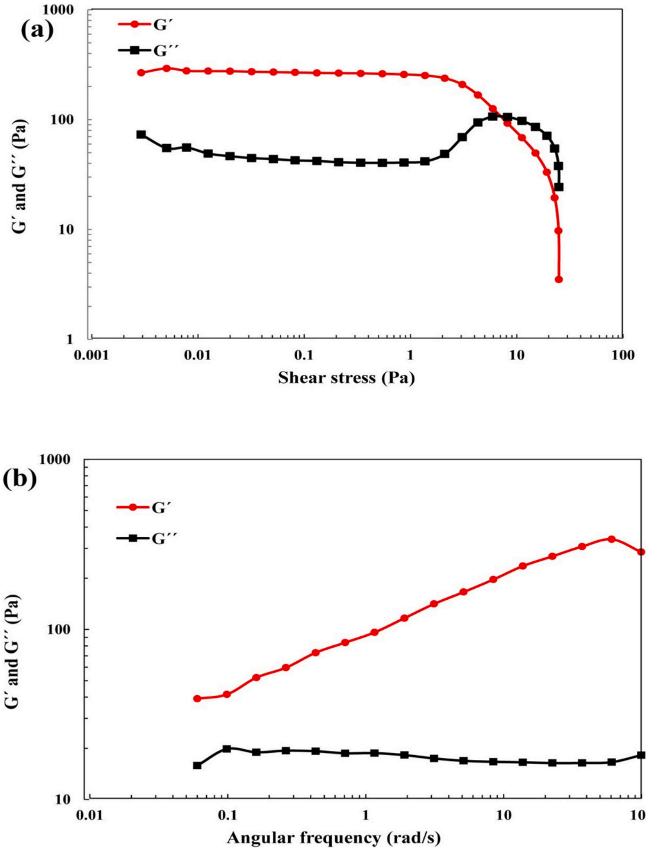

Fig. 3. (a) Amplitude and (b) frequency sweep curves of emulgel.

Table 2

Oxidation kinetic parameters of the initiation phase of emulsion and emulgel samples containing black seed oil.

Kinetic parameter	**IP (h)**	**$s_i \times 10^3$ (mM h^{-1})**	**AF**	**OR *10**	**A**	**$r_{AC0} \times 10^3$ (mM h^{-1})**	**$R_{eff} \times 10^3$ (h^{-1})**	**K_i/a (mM h^{-1})**
Emulsion								
Control	120.76 ± 5.33^{h}	22.10 ± 0.14^{cd}	–	–	–	–	–	
FA (1.03 mM)	249.12 ± 10.06^{fg}	10.70 ± 0.21^{efgh}	2.13 ± 0.05^{fg}	5.33 ± 0.29^{bcd}	4.80 ± 0.21^{ef}	4.15 ± 0.32^{gh}	0.22 ± 0.01^{b}	4.18 ± 0.35^{a}
FA (2.32 mM)	391.31 ± 8.60^{d}	8.65 ± 0.07^{fgh}	3.24 ± 0.03^{bcdefg}	4.05 ± 0.30^{cdef}	8.29 ± 2.11^{def}	6.06 ± 0.27^{gh}		
FA (3.61 mM)	573.06 ± 10.12^{bc}	6.80 ± 0.01^{gh}	4.90 ± 0.30^{abcde}	3.18 ± 0.30^{cdef}	15.34 ± 0.48^{cdef}	6.31 ± 0.45^{fgh}		
FA (4.89 mM)	601.55 ± 8.43^{bc}	7.00 ± 0.05^{gh}	5.11 ± 0.30^{abcd}	3.28 ± 0.40^{cdef}	15.66 ± 0.39^{cdef}	8.13 ± 0.11^{efg}		
FA (6.18 mM)	517.55 ± 27.77^{c}	9.40 ± 0.57^{efgh}	4.42 ± 0.34^{abcdef}	4.37 ± 0.30^{cde}	10.05 ± 0.75^{def}	11.96 ± 0.64^{de}		
GO (1.03 mM)	300.41 ± 15.00^{ef}	5.60 ± 0.28^{h}	2.54 ± 0.43^{efg}	2.62 ± 0.30^{cdef}	9.79 ± 0.88^{def}	3.43 ± 0.17^{gh}	0.20 ± 0.01^{b}	2.29 ± 0.01^{b}
GO (2.32 mM)	639.99 ± 10.01^{b}	2.70 ± 0.42^{h}	5.44 ± 0.26^{abc}	1.29 ± 0.40^{ef}	43.80 ± 7.89^{ab}	3.63 ± 0.06^{gh}		
GO (3.61 mM)	663.12 ± 32.89^{b}	4.35 ± 0.07^{h}	5.59 ± 0.32^{ab}	2.04 ± 0.49^{def}	27.79 ± 2.13^{bcd}	5.45 ± 0.30^{gh}		
GO (4.89 mM)	651.69 ± 20.98^{b}	4.40 ± 0.28^{h}	5.60 ± 0.34^{ab}	2.07 ± 0.40^{def}	26.92 ± 0.27^{bcde}	7.55 ± 0.86^{efgh}		
GO (6.18 mM)	576.17 ± 20.83^{bc}	17.20 ± 0.05^{def}	4.87 ± 0.10^{abcde}	8.05 ± 0.10^{ab}	6.11 ± 0.46^{def}	10.73 ± 0.39^{def}		
Emulgel								
Control	125.11 ± 3.57^{h}	51.10 ± 4.53^{a}	–	–	–	–		
FA (1.03 mM)	195.15 ± 5.98^{gh}	40.95 ± 2.01^{bc}	1.63 ± 0.40^{g}	8.10 ± 0.07^{ab}	2.16 ± 0.25^{f}	5.34 ± 0.80^{gh}		
FA (2.32 mM)	785.44 ± 3.75^{a}	6.80 ± 0.70^{gh}	6.45 ± 0.34^{a}	1.36 ± 0.07ef	57.10 ± 2.21^{a}	2.95 ± 0.01^{h}		
FA (3.61 mM)	311.64 ± 2.75^{def}	6.3 ± 0.89^{gh}	2.62 ± 0.03^{efg}	1.21 ± 0.02^{ef}	22.35 ± 2.97^{cdef}	11.83 ± 2.41^{de}	1.11 ± 0.17^{a}	1.30 ± 0.06^{c}
FA (4.89 mM)	196.67 ± 8.73^{gh}	4.60 ± 0.19^{h}	1.62 ± 0.44^{g}	0.89 ± 0.14^{f}	17.98 ± 2.03^{cdef}	24.89 ± 1.10^{b}		
FA (6.18 mM)	142.53 ± 4.49^{h}	41.10 ± 2.12^{bc}	1.19 ± 0.46^{g}	8.06 ± 0.29^{ab}	1.49 ± 0.63^{f}	43.98 ± 3.00^{a}		
GO (1.03 mM)	373.91 ± 24.56^{de}	27.90 ± 2.26^{c}	3.04 ± 0.49^{cdefg}	5.46 ± 0.04^{bc}	5.58 ± 0.94^{def}	2.76 ± 0.18^{h}		
GO (2.32 mM)	641.08 ± 4.49^{b}	8.15 ± 1.48^{gh}	5.25 ± 0.15^{abcd}	1.59 ± 0.15^{ef}	32.89 ± 1.31^{bc}	3.62 ± 0.03^{gh}		
GO (3.61 mM)	643.44 ± 0.91^{b}	15.50 ± 2.69^{defg}	5.28 ± 0.18^{abcd}	3.02 ± 0.26^{cdef}	10.88 ± 1.54^{cdef}	5.61 ± 0.01^{gh}	0.50 ± 0.07^{b}	1.17 ± 0.12^{c}
GO (4.89 mM)	346.49 ± 16.32^{de}	17.65 ± 0.35^{de}	2.93 ± 0.22^{defgh}	3.46 ± 0.24^{cdef}	8.60 ± 2.21^{def}	14.60 ± 2.10^{d}		
GO (6.18 mM)	308.40 ± 7.98^{def}	49.85 ± 1.09^{a}	2.55 ± 0.34^{efg}	9.91 ± 0.38^{a}	2.87 ± 0.16^{f}	20.08 ± 1.31^{c}		

[*] Mean ± SD ($n = 3$). In each section of each column, means with different superscript letters are significantly different ($P < 0.05$). F: ferulic acid, GO: γ-oryzanol. A: activity, AE: antioxidant effectiveness, IP: induction period, OR: oxidation rate ratio, r_{AC}: average rate of ferulic acid or γ-oryzanol consumption, s_i: pseudo-zero order rate constant, R_{eff}: rate constant of ferulic acid or γ-oryzanol consumption in side reactions of chain initiation, Ki/a: extent of ferulic acid or γ-oryzanol participation in side reactions of chain initiation.

peroxidation rate (Laguerre et al., 2017). In emulgels, the mobility of pro-oxidant and antioxidant compounds is limited (Keramat, Golmakani, et al., 2023; Sato, Moraes, & Cunha, 2014). Thus, the lower oxidation rate of emulgel sample than that of emulsion sample can be attributed to the limited accessibility of pro-oxidant in the aqueous phase to the unsaturated lipids in the oil phase.

The A values of FA and GO samples which simultaneously evaluates the OR value (ratio of FA or GO s_i value to the control sample s_i value) and AF value (ratio of FA or GO *IP* value to the control sample IP value) are presented in Table 2. FA and GO showed different antioxidant behavior in emulgel and emulsion samples. In emulgel samples, FA at 2.32, 3.61, and 4.89 mM concentration showed higher A values than those of GO. FA at 2.32 mM concentration exhibited the highest A value. The r_{AC0} value shows the mean rate of FA or GO consumption in emulgel and emulsion samples. The r_{AC0} values of FA at 1.03, 2.32, 3.61, and 6.18 mM concentration in emulgel samples were higher than the r_{AC0} values of GO in emulgel. In dispersed systems, the reaction between peroxyl radicals and antioxidant occurs at the interfacial layer. Thus, antioxidants that are able to concentrate at the interfacial layer can significantly inhibit peroxyl radical (). It has been reported that FA has high affinity for concentrating at interfacial layer. This can be related to the simultaneous presence of hydroxyl and carboxyl groups in FA chemical structure which can act as an important driving force for FA movement toward the interfacial layer. However, GO mainly concentrate inside the oil droplet. This can be due to the existence of phytosteryl group in GO which can enhance its solubility and concentration in the oil phase (Toorani & Golmakani, 2021). Therefore, the higher A value of FA than GO can be attributed to the higher ability of FA for concentrating at the interfacial layer.

GO at 1.03, 2.32, 3.61, and 4.61 mM concentrations showed lower A values in emulgel samples than those of emulsion samples. In addition, the r_{AC0} values of GO in emulgel samples were lower than those r_{AC0} values of GO in emulsion samples. The lower A values and r_{AC0} values of GO in emulgel can be explained by mass transport phenomena and limited ability of GO to transfer toward the interfacial layer in emulgel. In dispersed systems, it is expected that GO mainly concentrate in the oil phase due to its high hydrophobicity (lop P value of 10.12), while peroxyl radicals are amphiphilic molecules and concentrate near the interfacial area. In emulgels, movement of antioxidant compounds from the oil phase toward the interfacial layer is difficult (Keramat, Ehsandoost, & Golmakani, 2023; Keramat, Golmakani, et al., 2023). Therefore, GO can hardly move from the oil phase toward the interfacial layer and inhibit peroxyl radicals. Accordingly, the lower A values of GO in emulgel samples than those of emulsion samples can be attributed to the lower capacity of GO to transfer toward the interfacial layer and inhibit peroxyl radicals in emulgel. Alemán et al. (2015) showed that the physicochemical characteristics of different food emulsions can alter the antioxidant capacity of caffeic acid esters with different alkyl chain length. They observed that butyl, octyl, and dodecyl caffeates exhibited the highest antioxidant capacity in mayonnaise, while methyl and butyl caffeates exhibited the highest antioxidant capacity in milk emulsions. They stated that the mobility of antioxidants in mayonnaise is difficult due to the high viscosity of mayonnaise. Therefore, caffeic acid esters with medium chain length that are expected to concentrate near the interfacial layer would be more efficient than caffeic acid esters with short alkyl chain length that may need to transfer toward the interfacial layer. In contrast, the mobility of caffeic acid esters in milk emulsion is not limited due to the low viscosity of milk emulsion. Therefore, caffeic acid esters with short alkyl chain length can move easily toward the interfacial layer in milk emulsion and show high antioxidant activity (Alemán et al., 2015).

3.4. *Effect of FA and GO on the emulgel and emulsion oxidation kinetic in the propagation phase*

The TP value indicates the time when the production rate of LOOH reaches its maximum value. The P_o value indicates the oxidizability of emulsion and emulgel samples in the propagation phase (Farhoosh, 2021). The TP value of control emulsion sample was lower than that of control emulgel sample (Table 3). In addition, the P_o value of control emulsion sample was higher than that of control emulgel sample. In addition, the PP values of control emulgel and emulsion samples were 1059.92 and 679.27 h, respectively. Embedding emulsion droplets into the gel matrix enhanced the PP value and IP by 35.91 % and 3.48 %, respectively. Accordingly, the inhibitory effect of embedding emulsion droplets into the emulgel structure was more considerable in the propagation phase than that of initiation phase.

Table 3

Oxidation kinetic parameters of the propagation phase of emulsion and emulgel samples containing black seed oil.

Kinetic parameter	TP (h)	PO × 10^4 (h^{-1})	PP (h)	ET (h)
Emulsion				
Control	332.64 ± 16.69^{g}[*]	10.75 ± 1.06^{efgh}	679.27 ± 26.90^{ghij}	800.03 ± 0.57^{k}
FA (1.03 mM)	531.87 ± 22.76^{f}	13.88 ± 0.53^{bcd}	643.37 ± 33.76^{ij}	892.50 ± 12.30^{jk}
FA (2.32 mM)	777.19 ± 39.68^{e}	11.50 ± 1.06^{defg}	822.52 ± 40.60^{efghi}	1213.83 ± 0.59^{defgh}
FA (3.61 mM)	930.47 ± 54.47^{bcde}	13.38 ± 0.53^{bcde}	731.54 ± 29.22^{ijk}	$1304.60 \pm 69.31^{cdefg}$
FA (4.89 mM)	888.76 ± 5.53^{cde}	16.13 ± 0.18^{ab}	597.30 ± 6.30^{ij}	1198.86 ± 2.13^{efghi}
FA (6.18 mM)	802.74 ± 30.56^{e}	14.88 ± 0.18^{bc}	621.35 ± 6.78^{ij}	$1138.90 \pm 34.55^{fghij}$
GO (1.03 mM)	545.50 ± 1.81^{f}	12.38 ± 1.94^{cdef}	654.18 ± 23.72^{hij}	954.59 ± 32.47^{ijk}
GO (2.32 mM)	914.68 ± 20.66^{bcde}	17.75 ± 0.71^{a}	556.60 ± 21.89^{j}	$1196.59 \pm 31.89^{efghi}$
GO (3.61 mM)	1024.09 ± 52.29^{bc}	13.38 ± 3.36^{bcde}	746.97 ± 26.80^{efghi}	1410.09 ± 22.51^{cde}
GO (4.89 mM)	1002.66 ± 18.20^{bc}	13.50 ± 2.12^{bcde}	725.96 ± 6.78^{efghi}	1377.66 ± 40.73^{cdef}
GO (6.18 mM)	1009.73 ± 13.94^{bc}	11.12 ± 0.18^{defg}	883.05 ± 14.04^{defg}	1459.22 ± 6.80^{bcd}
Emulgel				
Control	634.30 ± 10.50^{f}	9.50 ± 2.83^{fghi}	1059.92 ± 13.53^{bc}	$1185.03 \pm 25.00^{efghi}$
FA (1.03 mM)	924.78 ± 6.07^{bcde}	6.25 ± 0.02^{k}	1529.63 ± 35.16^{a}	1724.78 ± 6.06^{a}
FA (2.32 mM)	1234.69 ± 0.90^{a}	10.50 ± 0.03^{fgh}	925.44 ± 3.75^{de}	1710.88 ± 7.01^{a}
FA (3.61 mM)	822.84 ± 43.85^{de}	8.75 ± 0.18^{ghij}	1082.63 ± 19.74^{bc}	1394.27 ± 43.85^{cdef}
FA (4.89 mM)	534.47 ± 17.35^{f}	10.25 ± 0.35^{fgh}	825.90 ± 13.20^{efgh}	1022.56 ± 11.41^{hijk}
FA (6.18 mM)	564.44 ± 22.52^{f}	10.00 ± 0.04^{fghi}	921.91 ± 15.15^{de}	1064.44 ± 39.02^{ghij}
GO (1.03 mM)	1028.95 ± 1.99^{bc}	7.38 ± 0.18^{jk}	1333.20 ± 42.81^{b}	1707.12 ± 18.25^{a}
GO (2.32 mM)	939.61 ± 13.37^{bcde}	15.63 ± 0.53^{ab}	618.72 ± 28.73^{fij}	$1259.80 \pm 24.24^{defgh}$
GO (3.61 mM)	1070.05 ± 50.56^{b}	10.38 ± 0.88^{fgh}	910.29 ± 36.08^{def}	1553.73 ± 41.25^{abc}
GO (4.89 mM)	911.11 ± 39.61^{bcde}	8.13 ± 0.53^{ijk}	1181.31 ± 46.32^{bc}	1527.81 ± 7.08^{abc}
GO (6.18 mM)	974.79 ± 16.61^{bcd}	7.25 ± 0.35^{jk}	1356.87 ± 70.37^{b}	1665.28 ± 50.28^{ab}

[*] Mean ± SD ($n = 3$). In each section of each column, means with different superscript letters are significantly different ($P < 0.05$). F: ferulic acid, GO: γ-oryzanol. ET: end time of propagation period, PO: propagation oxidizability, PP: propagation period, and TP: turning point.

The TP values of emulgel samples treated with GO at 1.03, 3.61, 4.89, and 6.18 mM concentrations were higher than those emulgel samples containing FA at equal concentration. In addition, the TP values of emulsion samples containing GO at all investigated concentration were higher than those samples containing FA at equal concentration. In the initiation phase, the r_{AC0} values of FA in both emulgel and emulsion samples were higher than those of GO. Therefore, higher amounts of FA consumed in the initiation phase and lower amounts of FA were active in the propagation phase, while lower amounts of GO were consumed in the initiation phase and higher amounts of GO were active in the propagation phase, resulting in the better performance of GO than FA in the propagation phase. In contrast, Toorani and Golmakani (2021), reported that FA showed higher antioxidant activity than GO in both initiation and propagation phases of sunflower oil oxidation. This indicates that the antioxidant activity of FA and GO in the initiation and propagation phase depends on the physicochemical properties of the lipid system.

3.5. *Antioxidant mechanism of FA and GO in emulgel and emulsion based on black seed oil*

The relationship between AF values and AC_0 values of FA and GO in emulsion and emulgel samples are shown in Fig. 4a and Fig. 4b, respectively. Also, the relationship between OR values and AC_0 values of FA and GO in emulsion and emulgel samples are shown in Fig. 4c and Fig. 4d, respectively. In both of the emulsion and emulgel samples, a nonlinear relationship was found between AF values and AC_0 values of FA and GO. This indicates non-ideal behavior of both FA and GO in emulsion and emulgel samples. In fact, nonlinear relationship between AF value and FA and GO concentration means that FA and GO molecules, besides taking part in reaction 7 (Fig. 2), which is the major termination reaction ($LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet}$), have taken part in side reactions of initiation chain. Reaction 11 ($LOOH + AH \rightarrow LO^{\bullet} + A^{\bullet} + H_2O$) and reaction 12 ($AH + O_2 \rightarrow A^{\bullet} + HOO^{\bullet}$) are side reactions of the initiation chain. Participation of FA and GO in these reactions results in production of alkoxyl radical ($LO^{\bullet}$) and hydroperoxyl radical ($HOO^{\bullet}$), which can enhance the peroxidation rate. On the other hand, a nonlinear relationship was found between OR values and AC_0 values of FA and GO in both of the emulsion and emulgel samples (Fig. 4c and Fig. 4d). This shows that FA and GO have participated in useful reactions such as reaction 8 ($A^{\bullet} + LOO^{\bullet} \rightarrow AOOL$), which can terminate the propagation chain (Toorani, Jokar, Nateghi, & Golmakani, 2024).

In both of the emulsion and emulgel samples, a linear relationship (mean R^2 of 0.97) between the r_{AH} values and AC_0 values at $n = 2$ was observed for FA and GO (Fig. 4e and Fig. 4f). This shows that FA and GA have taken part in both of the reactions 11 and 12. Toorani et al. (2020) reported that GO participated only in one of the reactions 11 or 12 in olive, rice bran, and canola oils. This indicates that the antioxidant pathways in which GO takes part in lipid systems depends on the physical and chemical properties of the lipid system.

The R_{eff} value (rate constant of FA and GO consumption in side reactions of the initiation chain) and K_i/a value (the extent of participating FA and GO molecules in reactions 10 and 11) of FA were higher than those of GO in both of the emulsion and emulsion samples (Table 2). Therefore, FA showed higher tendency than GO for reacting with LOOH and O_2. As mentioned in section 3.3, GO is expected to mainly locate in the oil phase due to its high hydrophobicity. Therefore, the lower R_{eff} value of GO can be related to its lower accessibility to the LOOH and O_2, which are expected to mainly concentrate in the interfacial layer and water phase, respectively.

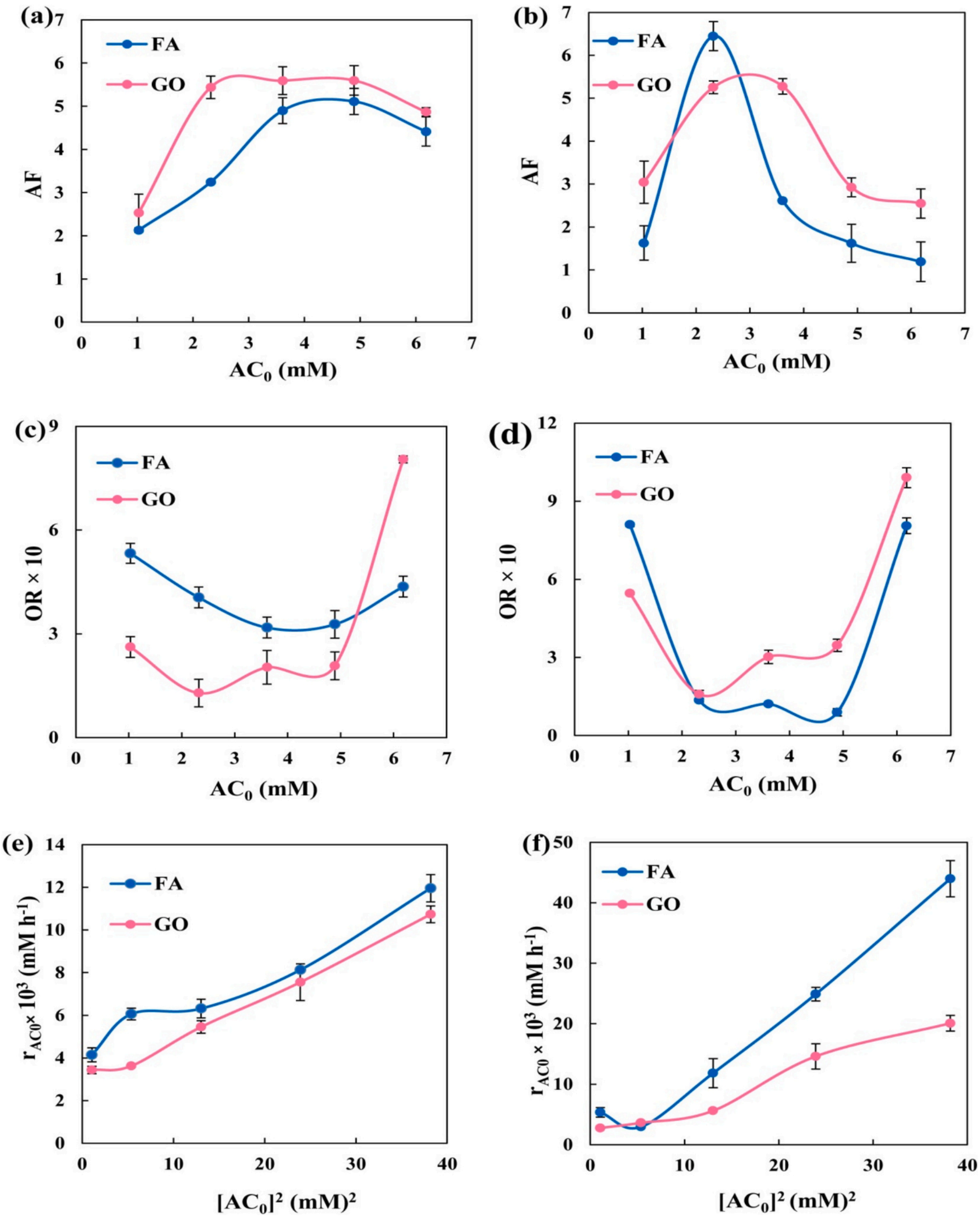

Fig. 4. Dependence of AF value (antioxidant effectiveness; a, b), OR value (oxidation rate ratio, c, d), and r_{AC0} value (average rate of ferulic acid or γ-oryzanol consumption; e, f) on the $[AC]_0$ (concentration of ferulic acid or γ-oryzanol) during emulsion (a, c, e) and emulgel (b, d, f) oxidation. FA: ferulic acid; GO: γ-oryzanol.

On the other hand, a nonlinear relationship was found between OR values and AC_0 values of FA and GO in both of the emulsion and emulgel samples (Fig. 4c and Fig. 4d). This shows that FA and GO have participated in useful reactions such as reaction 8 ($A^{\bullet} + LOO^{\bullet} \rightarrow AOOL$), which can terminate the propagation chain (Toorani et al., 2024).

The relationship between s_i values and AC_0 values of FA and GO are illustrated in Fig. 5. In both of the emulsion and emulgel samples, a nonlinear correlation was found between s_i values and AC_0 values of FA and GO at $n = -0.5$ and $n = -1$. This means that FA and GO radicals have participated in more than one chain propagation reaction. The free radicals produced in these reactions can enhance the peroxidation rate in emulsion and emulgel samples (Toorani et al., 2024). Similarly, Keramat and Golmakani (2024a) showed that curcumin and curcumin esters radicals participate in more than one chain propagation reaction in oleogel and emulgel containing linseed oil.

4. Conclusion

The results of this research indicated that FA and GO exhibited different antioxidant behavior in emulgel and emulsion. In the initiation phase, FA showed higher A value than that of GO in emulgel samples and the average rate of FA consumption in emulgel was higher than that of GO. However, in the propagation phase, GO showed higher A value than FA in both of the emulsion and emulgel samples. This can be attributed to the consumption of lower amounts of GO in the initiation phase and presence of higher amounts of active GO than that of FA in the propagation phase. The control emulgel sample showed lower oxidation rate than that of control emulsion sample. However, the inhibitory effect of

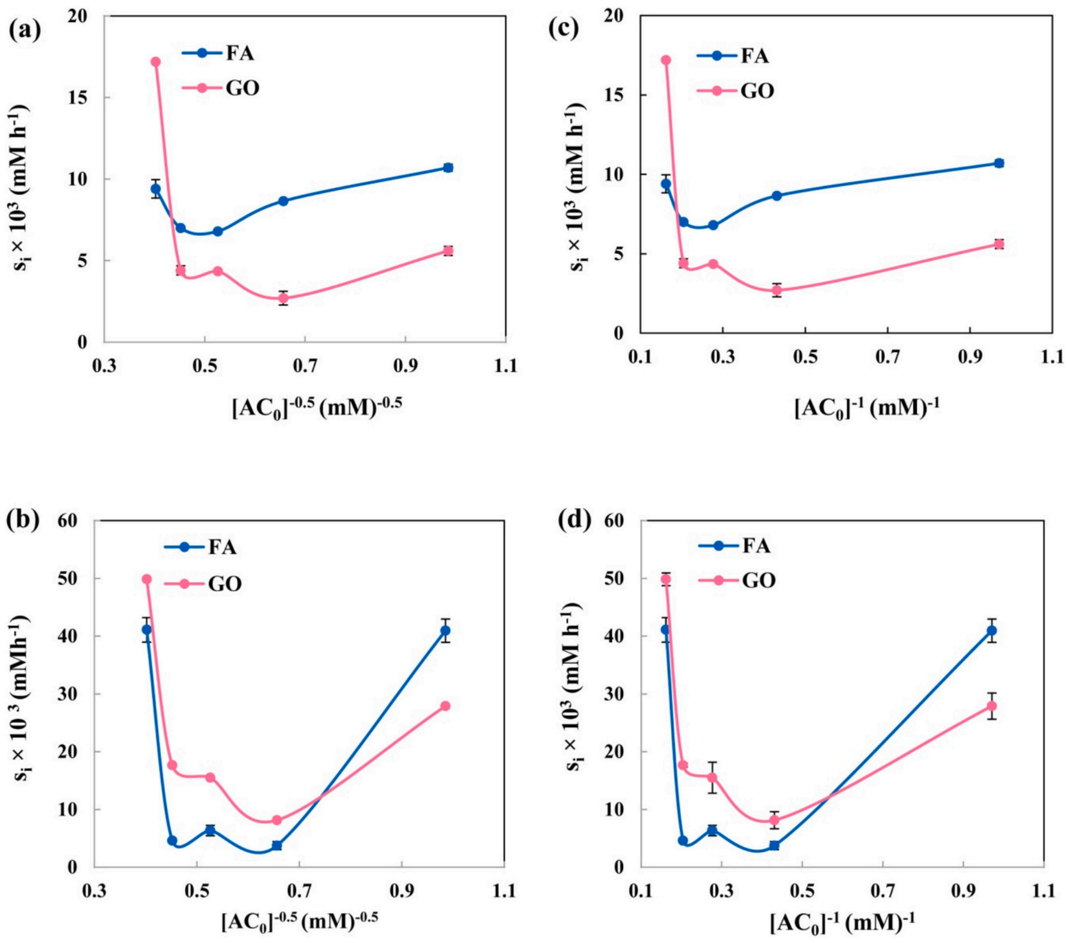

Fig. 5. Dependence of the s_i value (pseudo-zero order rate constant) on the $[AC]_0$ (ferulic acid or γ-oryzanol) during emulsion (a, c) and emulgel (b, d) oxidation. FA: ferulic acid; GO: γ-oryzanol.

embedding emulsion droplets into the emulgel structure was more considerable in the propagation phase than that of the initiation phase. Investigating the antioxidant mechanism showed that both GO and FA exhibited non-ideal behavior in emulsion and emulgel samples. In addition, FA and GO radicals were active in both of the emulsion and emulgel samples and participated in more than one chain propagation reaction.

CRediT authorship contribution statement

Asma Rasekhi Kazeruni: Methodology, Investigation, Formal analysis, Data curation. **Seyed Mohammad Hashem Hosseini:** Supervision, Project administration, Funding acquisition. **Malihe Keramat:** Writing – original draft, Visualization, Validation, Software, Conceptualization. **Mehrdad Niakousari:** Supervision. **Fatemeh Ghiasi:** Supervision. **Mohammad-Taghi Golmakani:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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