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Author Contributions

Ruijie Liu and Mengyao Lu designed the study and interpreted the results. Mengyao Lu, Tao Zhang, Zhongrong Jiang, Yiwen Guo, Fangcheng Qiu, Ruru Liu, Lisha Zhang, Ming Chang, Qingzhe Jin, Xingguo Wang executed the research. All authors contributed writing and approved the final version of the manuscript.

Physical Properties and Cellular Antioxidant Activity of Vegetable Oil Emulsions with Different Chain Lengths and Saturation of Triglycerides

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Abstract

Lipid concomitants are the main trace substances that determine the oxidative stability of oils. Over 90% of oils are composed of triglycerides, which affect oxidative stability. Oils typically enter living organism as emulsions. This study investigated the physical properties and cellular antioxidant activity (CAA) of emulsions in two model oils, namely, rice bran oil (RBO) with long-chain unsaturated triglycerides and coconut oil (CNO) with medium-chain saturated triglycerides. The results were as follows: the mean particle sizes were all approximately 250 nm; the polydispersity index (PDI) values were below 0.2; and the zeta potential values were more negative than -30 mV. The droplet size distribution of CNO emulsions was more concentrated than that of RBO emulsions. Rice bran oil-S (RBO-S) emulsions exhibited the highest CAA value, but the CAA value of coconut oil-C (CNO-C) emulsions was higher than that of rice bran oil-D (RBO-D) emulsions, indicating that CAA was influenced by the combined effect of triglycerides and minor constituents. Moreover, the medium-chain saturated triglycerides in vegetable oil emulsions resulted in a stronger CAA than that of long-chain saturated triglycerides.

***Keywords*:** Emulsion; Triglycerides; Cellular antioxidant activity; Rice bran oil; Coconut oil

1. Introduction

In recent years, research on whole food, containing a naturally occurring a mixture of phytochemicals and antioxidant compounds, for the amelioration of oxidative status, not a single compound, has received widespread attention (Johar et al, 2018). Vegetable oils contain abundant and biologically active minor constituents, such as polyphenols, sterols, oryzanol, and tocopherols (Siger, Nogala-Kalucka, & Lampart-Szczapa, 2008; Kozłowska, Gruczyńska, Ścibisz, & Rudzińska, 2016), and they also contain 90-98% glycerides, are mainly composed of different chain lengths and saturation of triglycerides (Rao, 2001; Yalagala, Sugasini, Ramaprasad, & Lokesh, 2017). Although minor constituents have been always used to indicate the antioxidant property of vegetable oils (Szydlowska-Czerniak & Laszewska, 2015), the oil itself, such as the type of triglycerides, may affect the overall measurement result. (Pálová, Charvat, & Kvapil, 2008). In addition, unsaturated fatty acids with double bonds are easily attacked by free radicals (Meroni & Raikos, 2017). Peony seed oil possesses a potent scavenging effect against free radicals as well as in vivo antioxidant capacity, which is related to fairly low ratio of n-6 to n-3 polyunsaturated fatty acids originating from its uncommon abundance in α-linolenic acid (Yang et al., 2017). Phenolic compounds display different properties in different plant matrices (Aalim, Belwal, Wang, Luo, & Hu, 2018). Therefore, different triglycerides systems may affect the antioxidant activity of the active compounds in vegetable oils. Moreover, some functional foods are developed based on combinations of different vegetable oils, such as in the preparation of *trans*-free structure lipids (Lakum &

Sonwai, 2018), and the production of saturated fat-reduced processed cheese products based on lipid composition. The principle may involve the differences in the interaction between the fatty acids of vegetable oils and the protein network structure (Huang, Hallinan, & Maleky, 2018). In processed foods, vegetable oils are mostly stabilized in the form of emulsions, which improve the function and quality of the oil. Whether consumed as a whole food or edible oil, the intake, absorption, and metabolism of oil in the body are completed in various emulsion forms as lipolysis takes place at the oil-water interface (Drewnowski & Almiron-Roig, 2010; Gallier & Singh, 2012). It has been previously reported that the type and polarity of the lipid phase in emulsions significantly affect the antioxidant activity of active compounds (Hopia, Huang, Schwarz, German, & Frankel, 1996). Therefore, it is important to investigate the effects of triglycerides with different chain lengths and saturation on the antioxidant capacity of whole vegetable oils in the form of emulsions, which represents the form that the body intakes.

Emulsions allow incorporation of high nutritional value oils, such as high-oleic palm oil (Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal, 2016*)*, linseed oil (Sotomayor-Gerding et al., 2016) or clove essential oil, with high in vitro bactericidal action, thus improving their bioavailability (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martin-Belloso, 2015). Emulsions improve the functionalities of vegetable oils due to reduced droplet size, which increases the specific surface area. Emulsions are kinetically stable colloidal dispersions that aid oils to overcome water insolubility and instability, simultaneously enhancing cellular uptake of oils (Solans,

Izquierdo, Nolla, Azemar, & Garcia, 2005; Ma, Yu, Yin, Tang, & Yang, 2018). There are many factors that affect emulsion properties, such as environmental conditions (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martin-Belloso, 2015), system components (including different oils composed of various triglycerides with different chain lengths and saturation degrees), and emulsifier type. One of the commonly used emulsifiers in the formation and stabilization of emulsions is whey protein isolate (WPI), which is natural and adsorbs more easily at the oil-water interface, thus preventing droplets from aggregating by generating repulsive interactions and forming stabilizing emulsions during homogenization (Ozturk, Argin, Ozilgen, & McClements, 2015; Li et al, 2019).

The following chemical assays are often used to determine the antioxidant ability of oil extracts: oxygen radical absorbance capacity (ORAC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and 2, 2'-azino-bis, 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) (Shi, Zheng, Jin, & Wang, 2017; Liu et al., 2019). However, the cellular antioxidant activity (CAA) assay is a common model for evaluating antioxidant function at a biological level. Measurement of the ability of compounds to prevent the formation of fluorescent dichlorofluorescein by 2,2′ -azobis (2-amidinopropane) dihydrochloride (ABAP)-generated peroxyl radicals in HepG2 cells allows quantification of the CAA of dietary supplements, foods and phytochemicals. This reaction of cellular biochemical processes includes uptake, distribution, bioavailability and metabolism of the antioxidant components (Wolfe & Liu, 2017; Liu et al., 2019).

Rice bran oil (RBO) is a type of nutritionally rich oil, mainly composed of long-chain unsaturated fatty acids. The fatty acid composition of RBO is mainly composed of oleic, linoleic, and palmitic acids. RBO contains γ-oryzanol, tocopherols, and sterols, which have many bioactive abilities (Friedman & Mendel, 2013; Piriyaprasarth, Juttulapa, & Sriamornsak, 2016). Coconut oil (CNO) is a rare vegetable oil, mainly composed of medium-chain saturated triglycerides, which are easily absorbed into the body and stable against oxidation, thus not prone to peroxide formation (Bhatnagar, Prasanth Kumar, Hemavathy, & Gopala Krishna, 2009).

In this study, RBO and CNO were selected as typical oils with different types of triglycerides, and the CAA model was exploited to compare the cellular antioxidant properties of whole oil in the form of emulsions. We aimed to explore the effects of different triglyceride systems on the cellular antioxidant properties of whole vegetable oil emulsions, which may provide a theoretical reference for the combination of triglycerides and minor constituents from the perspective of the antioxidant property of the vegetable oil.

2. Material and methods

2.1 Samples and materials

2.1.1 Samples

The following rice bran oils were used: RBO-D (Zhejiang), RBO-P (Jiangsu), and RBO-S (Jiangsu). The following coconut oils were used: CNO-C (Shanghai) and

CNO-J (Philippines). Rice bran oils (RBOs) and coconut oils (CNOs) from different origins were all purchased from supermarkets.

2.1.2 Materials and chemicals

Whey protein isolate (WPI; Cheshire, UK, CW97RA) was purchased from Hilmar Food International, Inc. (Livingston, CA). Standard tocopherols (α-, β-, γ- and δ-isomers; purity>95%), oryzanol, 5α-cholestane, 2′, 7′-dichlorofluorescin diacetate (DCFH-DA), methylene blue dye solution, quercetin, and glutaraldehyde solution FL were obtained from Sigma-Aldrich Chemical Co. (Shanghai, China). 2,′ -azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals Co. (Shanghai, China). Hanks' Balanced Salt Solution (HBSS), Dulbecco's minimum EM (DMEM), foetal bovine serum (FBS), streptomycin, penicillin, and phosphate-buffered saline (PBS) solution were obtained from Gibco Life Technologies (Grand Island, NY). Folin-Ciocalteu (FC) reagent, sodium carbonate, acetic acid, methanol, ethanol, and other chemicals were obtained from Chemical Reagent Company (Shanghai, China). HepG2 cells were acquired from the American Type Culture Collection (Rockville, MD).

2.2 Determination of RBO and CNO composition

2.2.1 Determination of triglycerides and fatty acids composition

The equivalent carbon number (ECN) of triglycerides was detected by HPLC-ELSD. Briefly, 20 μL of sample was injected at flow rate of 0.8 mL/min. Mobile phase A was acetonitrile, and mobile phase B was isopropanol. The following

143 elution procedure was performed: 0-30 min, 70% A/30% B linearity becomes 60%
144 A/40% B; 30-70 min, 60% A/40% B linearly becomes 55% A/45% B; 70-90 min, 55%
145 A/45% B is kept constant; and 90-95 min, linearly changed to 70% A/30% B. The
146 area normalization method was used for quantification. Fatty acid composition was
147 determined using a GC-14B gas chromatography (Shimadzu, Tokyo, Japan) equipped
148 with a flame ionization detection (FID) (Agilent) as previously described (Amaral,
149 Cunha, Alves, Pereira, Seabra, & Oliveira, 2004) with the following parameters: FID
150 temperature of 250 °C; inlet temperature of 250 °C; split ratio of 1:50; temperature
151 programming process at 80 °C for 3 min followed by 15 °C/min to 215 °C and hold
152 20 min; acquisition time of 30 min; and injection volume of 2.0 μL.

153 *2.2.2 Determination of minor constituent content*

154 Tocopherols were determined using high-performance liquid chromatography
155 (HPLC) (LC-20AT; Shimadzu, Kyoto, Japan) (Gao, Jin, Liu, Jin, & Wang, 2018).
156 The following parameters were used: column temperature of 30 °C; mobile phase of
157 hexane/isopropanol (98.5/1.5, *v/v*); rate of 1.0 mL/min; and determining wavelength
158 at 295 nm. Oryzanol was determined using HPLC with the following parameters:
159 column temperature of 40 °C; mobile phase of ethanol; rate of 0.8 mL/min; and
160 determining wavelength at 324 nm (Liu et al., 2019). Sterols were determined using a
161 gas chromatography-mass spectrometry (GC-MS) system (Thermo Fisher) equipped
162 with a FID (Thermo Fisher) following the method of Jin et al (2016). The total
163 phenolic content of the oil extracts was measured using the Folin-Ciocalteu method
164 (Gómez Caravaca, Carrasco Pancorbo, Cañabate Díaz, Segura Carretero, &

Fernández Gutiérrez, 2005). Oil (1.5 g) was dissolved in 6 mL of hexane and was passed through a Diol-SPE column, leaving the sample on the solid phase. The column was washed with two portions (of 3 mL) of hexane. Finally, the sample was recovered by passing through 6 mL of methanol to extract phenolic compounds from oils.

2.3 Preparation of emulsions

Emulsions were prepared according to a previously reported method (Ozturk, Argin, Ozilgen, & McClements, 2015) with minor adjustments. Emulsions were prepared by high pressure homogenizing 10% (*w/w*) of lipid phase (RBO or CNO) with 90% (*w/w*) of aqueous phase. The aqueous phase consisted only of 1% (*w/v*) emulsifier (WPI), which was stirred at room temperature overnight (10-12 h) to fully hydrate. Coarse emulsions were obtained by blending both oil and protein solution together using a high-speed blender (T25, IKA, Germany) for 2 min at 19000 rpm, and they were passed through a high-pressure homogenizer (01C100-1K0, GEA, Germany) for 60 MPa and 3 cycle times to gain proper emulsions.

2.4 Determination of emulsion physical properties

Based a previous report (Galvao, Vicente, & Sobral, 2017), the mean particle size, PDI, and droplet size distribution of RBO and CNO emulsions were measured by dynamic light scattering (DLS), and the zeta potential was determined by phase-analysis light scattering (PALS). All determinations were made using a multi-angle particle size and high sensitivity zeta potential analyser (Nano Brook

Omni; Brookhaven Instruments, USA). The mean particle size of each emulsion was represented as the surface-weighted mean diameter, which was calculated from the full particle size distribution. The refractive index of oil droplets and water phase were set to 1.450 and 1.330, respectively. To avoid multiple scattering influences, the emulsions were diluted 100-fold with ultrapure water before measurement. The measurements were performed at a fixed angle of 90° and were performed in triplicate.

2.5 Cell culture

HepG2 cells were cultured in growth medium containing DMEM/FBS/antimycotic solution (90:10:1, *v/v/v*) and incubated at 37 °C and 5% CO_2. Cells used were between passages 26 and 35.

2.6 Cytotoxicity analysis

Following a previously reported method (Wolfe & Liu, 2017), the methylene blue assay was used to assess the potential toxic effects of the RBO and CNO emulsions on HepG2 cells. HepG2 cells were seeded at 3×10^4/well in a 96-well plate in 100 μL of growth medium and incubated at 37 °C for 24 h. Emulsion treatments in 100 μL of medium were applied to cells followed by incubation at 37 °C for 24 h. A volume of 50 μL/well of methylene blue staining solution (98% HBSS, 0.67% glutaraldehyde, and 0.6% methylene blue) was applied to each well followed by incubation at 37 °C for 1 h. After washing the wells using water, 100 μL of elution solution (49% PBS, 50% ethanol, and 1% acetic acid) was added to each well, and the plate was placed on a

bench-top shaker for 20 min to allow uniform elution. Absorbance was read at 570 nm using the Multiskan Go microplate reader (Thermo Scientific, USA). Emulsions were not considered to be cytotoxic if their absorbance was >90% compared to the control.

2.7 Cellular antioxidant activity assay

The CAA assay was used to assess the cellular antioxidant ability of the RBO and CNO emulsions in HepG2 cells following a previously reported procedure (Wolfe & Liu, 2017). HepG2 cells were seeded at 6×10^4/well in a 96-well black microplate with clear bottom, and 100 μL of medium was added to every well. The plate was incubated at 37 °C and 5% CO_2 for one day. Medium was then discarded, and wells were washed thoroughly with PBS solution. Medium (100 μL) was then added with corresponding concentrations of oil emulsions or controls containing DCFH-DA (50 μM) into triplicate wells. After incubation, the solution was removed, and cells were washed with PBS. Finally, 100 μL of HBSS containing ABAP (600 μM) was added into the wells, and the microplate was placed into a Fluoroskan Ascent FL microplate reader (Thermo Scientific, USA). Fluorescence at the excitation and emission wavelengths of 535 nm and 485 nm, respectively, was measured at 37 °C every 5 min (13 times in total).

CAA (units) was calculated according to the proportions under the fluorescence against time curve according to the following formula:

$$\mathrm{CAA}(\%) = 100 - \frac{\int \mathrm{SA} - \int \mathrm{BA}}{\int \mathrm{CA} - \int \mathrm{BA}} \times 100$$

where $\int SA$, $\int CA$ and $\int BA$ are the integral areas under the fluorescence against time curve of the sample, control and blank, respectively.

The median effective dose (EC_{50}) was determined for the oil emulsions according to the median effect plotting of log (fa/fu) versus log (dose), in which fa is the part affected (CAA unit) and fu is the part unaffected (100-CAA unit). The EC_{50} values were expressed as μmol quercetin of per 100 g of oil (μmol QE/100 g).

2.8 Statistical Analyses

All analyses were performed in triplicate and expressed as the mean ± standard deviation (mean ± SD). SPSS version 20.0 was used to analyse variance, and significant differences determined by ANOVA were significant at the 5% level (p<0.05). Duncan's post-hoc tests were performed using SPSS version 20.0.

3. Results and discussion

3.1 Composition of RBO and CNO

3.1.1 Triglycerides and fatty acids composition

The five oils used for the study were analysed for fatty acid composition and ECN as shown in Table 1. The compositions of fatty acids in RBO samples were myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), and arachidic acid (C20:0), which had no significant differences (p>0.05). The data analysed here agreed with a previous literature report (Gopala Krishna, Hemakumar, & Khatoon, 2006).

Moreover, the unsaturated fatty acids content reached 80%. ECN is defined as the carbon number of the hypothetical saturated triglyceride, which elutes at the same retention time as the unsaturated triglyceride being studied (Podlaha & Bengt, 2015). The ECN of RBO samples, which also had no significant differences (p>0.05), were 42, 44, 46, and 48. However, the ECN of CNO samples were 36, 38, and 34. In addition, the CNO had highest content of lauric acid (C12:0; reached 46%) followed by myristic acid (14:0) and palmitic acid (16:0). The content of saturated fatty acids (SFA) in CNO reached 90%, which was consistent with a previous literature report (Bhatnagar, Prasanth Kumar, Hemavathy, & Gopala Krishna, 2009).

3.1.2 Minor constituent levels

The minor constituent analyses results presented in Table 2 showed that the RBOs all had oryzanol from 1543 ± 26 to 25611 ± 2571 mg/kg oil, but the CNOs did not contain oryzanol. The RBO samples had the same types of minor constituents but had significant differences in content (p<0.05). According to a previous study (Schwartz, Ollilainen, Piironen, & Lampi, 2008), the tocopherol contents of coconut oil are the lowest compared to other oils, which was consistent with the present result. The content of phytosterol in RBO was higher than that in CNO, and the content of β-sitosterol was higher in RBO than CNO. The polyphenols in CNO-J could not be detected. The content of total phenolic compounds was low but the content of oryzanol was high, which might be due to the incomplete extraction of oryzanol by methanol. Based on the above analyses, minor constituent contents in CNO were significantly lower than those of RBO (p<0.05).

3.2 Emulsion characterization

3.2.1 Mean particle size, PDI, and zeta potential

The results of the emulsion characterizations are shown in Figure 1. The mean particle sizes were all approximately 250 nm as shown in Fig. 1(a). The mean particle sizes of the RBO-S, CNO-C, and CNO-J emulsions had no significant difference (p>0.05), but the three RBO emulsions showed significant differences (p<0.05), indicating the different degree of influence of different oils on the physical properties. PDI represents a dimensionless measurement of the drop size distribution amplitude (Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal, 2016). The emulsions exhibited a PDI value below 0.2 with no significant difference among the oils (p>0.05) as shown in Fig. 1(b). These results indicated that the polydisperse particles are somewhat suitable (Yen, Wu, Lin, Cham, & Lin, 2008).

The surface charge of the droplets in the emulsion containing RBO and CNO is shown in Figure 1(c). There was no significant difference (p>0.05) of zeta potential among RBO-S, CNO-C, and CNO-J emulsions. In addition, the zeta potentials of the oil emulsions were more negative than -30 mV. These values are generally considered to be stable, indicating a strong electrostatic repulsion of the dispersed oil droplets in the aqueous phase, which were predominant in the emulsions (Heurtault, Saulnier, Pech, Proust, & Benoit, 2003). There was a difference in the zeta potential of emulsions prepared from different oils, which may be due to the differences between dissociation degree of the emulsifier and the amount of ionizable compounds of the oils, thereby affecting the adsorption of the surface-active compound at the two-phase

interface (Bonilla, Atarés, Vargas, & Chiralt, 2012; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martin-Belloso, 2015). RBO emulsions with long-chain unsaturated triglycerides had a more negative zeta potential than that of CNO emulsions, which may be related to a higher number of negatively charged particles (Celus, Salvia-Trujillo, Kyomugasho, Maes, Van Loey, Grauwet, & Hendrickx, 2018). The pH value of the emulsion (6.5) was higher than the pH value of the fatty acid (4.8-5), resulting in ionization of its carboxyl group. The free fatty acid in RBO was more easily ionized, resulting in a more negative charge. For anionic polymers, smaller particles have higher mobility than larger particles, allowing smaller electrophoretic particles to move faster (Celus, Salvia-Trujillo, Kyomugasho, Maes, Van Loey, Grauwet, & Hendrickx, 2018), which may explain the smaller average particle size but more negative potential value of the RBO-P emulsion. The stability of the emulsions was measured with droplet size and zeta potential (Ricaurte, Perea-Flores, Martinez, & Quintanilla-Carvajal, 2016).

3.2.2 Droplet size distribution of oil emulsions

The results of the droplet size distribution are shown in Figure 1(d). The different emulsions had a slight effect on the mean particle size. Intensity (%) represents the relative strength of the measured intensity of droplets of different particle sizes in the emulsion system, and the measured intensity shows that the strongest particle size value is close to the average particle size. The particle size distributions of RBO-D, RBO-P, and RBO-S were unimodal with gradual widening. However, the particle size distributions of CNO-C and CNO-J were unimodal and narrow. Thus, the droplet size

distribution of CNO emulsions was better than that of RBO emulsions. The final droplet size of the emulsion was the result of complex interactions among processing conditions, emulsifiers, and oil droplet adsorption (Wooster, Golding, & Sanguansri, 2008). It has been reported that the molecular characteristics, such as molecular weight, polarity and concept, of oil are important factors in determining the ability to bind to surfactants (Djekic & Primorac, 2008). Oils with higher concentrations of polar components may reduce the interfacial tension and result in droplet breakage during homogenization, which allows the oils to dissolve in the aqueous phase, resulting in a particle size distribution intensity peak of larger droplets (Ziani, Fang, & Mcclements, 2012). RBO had more abundant minor constituents with strong polarity than CNO. RBO-S, which was rich in minor constituents, had a wider intensity peak as shown in Figure 1(d). RBO was rich in long-chain triglycerides with larger molecular weight than CNO. CNO had medium-chain triglycerides, which may explain why RBO was more prone to form larger droplets. Because the emulsions were produced under the same conditions, their droplet size distribution results were related to the composition of the oil.

3.3 Cytotoxicity effects and antioxidant properties

In this study, the CAA assay using ABAP-induced peroxyl radicals in HepG2 cells was conducted to quantify the cellular antioxidant ability of oil emulsions by preventing the formation of DCF from oxidation determined by the decrease of fluorescence (Meng et al., 2017). As shown in Figure 2, the kinetics of DCFH

335 oxidation in HepG2 cells by peroxyl radicals generated from ABAP was measured for
336 oil emulsions. The increase in fluorescence due to the DCFH oxidation was inhibited
337 by oil emulsions in a dose-dependent manner as demonstrated by the curves generated
338 from HepG2 cells treated with five oil emulsions. These results were consistent with
339 those observed for Chinese hawthorn (Wen et al., 2015) and black tea (Liu & Huang,
340 2015).

341 Dose-response curves from the ratio of the area under the curve of the oil emulsion
342 sample to that of the blank and control indicated the inhibition of peroxyl
343 radical-induced DCFH oxidation by the five oil emulsions (Fig. 3). The R^2 values
344 were all greater than 0.9, indicating good dose-effect relationships. To calculate the
345 corresponding EC_{50}, the median effect curve was plotted for each oil emulsion (Fig.
346 4). The EC_{50} of quercetin, as the equivalent weight, was 4.91 μM (R^2 = 0.9841) in this
347 study.

348 Table 3 shows that HepG2 cell proliferation was >90% for 0-20 mg/mL (RBO-S),
349 0-50 mg/mL (RBO-P), greater than 20 mg/mL (CNO-C), and 40 mg/mL (RBO-D and
350 CNO-J). Therefore, we used these results as references to determine the CAA value
351 within the safe concentration range of various samples. The EC_{50} of RBO emulsions
352 and CNO emulsions were 5.9 ± 0.2 mg/mL (RBO-D), 4.6 ± 0.3 mg/mL (RBO-P), 0.6
353 ± 0.1 mg/mL (RBO-S), 4.6 ± 0.3 mg/mL (CNO-C), and 6.4 ± 0.2 mg/mL (CNO-J).
354 The intra experimental coefficient of variation (CV) for the RBO-S reached 16.7%
355 and was significantly higher than the CV of the other four samples, which were below
356 10%. The CAA unit of these five oil emulsions from high to low were 846.6 ± 0.6

μmol of QE/100 mg oil (RBO-S) > 107.2 ± 0.2 μmol of QE/100 mg oil (CNO-C) ≈ 106.1 ± 0.4 μmol of QE/100 mg oil (RBO-P) > 83.7 ± 0.4 μmol of QE/100 mg oil (RBO-D) > 76.7 ± 0.2 μmol of QE/100 mg oil (CNO-J) (Table 3). These results indicated that RBO-S showed the highest antioxidant ability against the ABAP-generated peroxyl radicals by preventing the oxidation of DCFH and membrane lipids, which decreased the formation of DCF in HepG2 cells. CNO-C and RBO-P had the next highest antioxidant abilities but showed no significant differences (p>0.05).

Both RBO-D and RBO-P emulsions exhibited significantly lower cellular antioxidant ability than RBO-S emulsions (p<0.05), which may have been due to the large amount of minor constituents in RBO-S, especially polyphenols. The CAA value of the CNO-C emulsion was close to that of the RBO-P emulsion but higher than that of the RBO-D emulsion, indicating that medium-chain and saturated triglycerides improve the uptake and bioavailability of antioxidant ingredients into HepG2 cells (Fan, Liu, Gao, Zhang, & Yi, 2018). Oil composed of medium-chain triglycerides is more readily digested and absorbed than oil composed of long-chain triglycerides (Takeuchi, Sekine, & Seto, 2008). In addition to the influence of the surface ultrastructure of the cell, transporter mRNA expression or enzyme activity, fatty acids with different chain lengths and saturation have different regulatory pathways. For example, long-chain fatty acids (stearic acid and *trans*10, *cis* 12 conjugated linolenic acid) tend to regulate extracellular matrix-receptor interactions (Yan, Tang, Zhou, Han, & Tan, 2019). Moreover, it has been reported that

379 polyunsaturated fatty acids have a significant effect on cell membrane fluidity, while
380 palmitic acid does not affect membrane fluidity (Nano, Nobili, Girard-Pipau, &
381 Rampal, 2003). The distributions of polyunsaturated fatty acid and minor constituents
382 in triacylglycerol may affect the functions of vegetable oils (Yalagala, Sugasini,
383 Ramaprasad, & Lokesh, 2017). In the polyunsaturated fatty acid emulsion system,
384 such as in the RBO emulsions, high concentration of α-tocopherol self-assembles to
385 form micelles, which reduces the tocopherol content at the oil-water interface and
386 reduces its antioxidant capacity (Huang, Frankel, Schwarz, & German, 1996).
387

388 **4. Conclusion**

389 Different types of triglycerides affected the emulsion physical properties. Oils with
390 medium-chain saturated triglycerides easily formed emulsions with concentrated
391 droplet size distribution. In contrast, oil emulsions rich in long-chain unsaturated
392 triglycerides showed a more dispersed droplet size distribution.

393 Different triglycerides also affected the cellular antioxidant properties of the whole
394 oil emulsion. The same vegetable oils, containing the same triglycerides, had different
395 cellular antioxidant capacity due to the composition and content of the minor
396 constituents. Medium-chain and saturated triglycerides in vegetable oil emulsions
397 resulted in strong CAA despite the lower amounts of minor constituents. The result of
398 the CAA was due to the combined effects of triglycerides and minor constituents of
399 the vegetable oil, which also demonstrated the necessity to study the antioxidant
400 properties of whole oils and whole foods.

401 Our next study on the cellular antioxidant properties of vegetable oils influenced by
402 triglycerides with different carbon chain lengths or different saturation will provide
403 additional knowledge when choosing more typical vegetable oils.
404

Conflicts of interest

There are no interests to declare.

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614

Table 1 Fatty acid, triglyceride composition of five vegetable oils.

	RBO-D	RBO-P	RBO-S	CNO-C	CNO-J
Fatty acid composition (%)					
C6:0	-	-	-	0.34 ±0.01 [a]	-
C8:0	-	-	-	5.59 ± 0.02 [a]	-
C10:0	-	-	-	5.20 ± 0.37 [a]	-
C12:0	-	-	-	46.78 ± 0.28 [a]	-
C14:0	0.26 ± 0.01 [a]	0.27 ± 0.01 [a]	0.23 ± 0.01 [a]	19.97 ± 0.22 [b]	0.26 ± 0.01 [a]
C16:0	17.04 ± 0.26 [b]	17.02 ± 0.13 [b]	17.16 ± 0.01 [b]	10.29 ± 0.07 [a]	17.04 ± 0.26 [b]
C16:1	0.15 ± 0.00 [a]	0.12 ± 0.00 [a]	0.12 ± 0.01 [a]	-	0.15 ± 0.00 [a]
C18:0	1.29 ± 0.02 [a]	1.39 ± 0.01 [a]	1.42 ± 0.01 [a]	3.26 ± 0.03 [b]	1.29 ± 0.02 [a]
C18:1	41.89 ± 0.15 [b]	41.95 ± 0.07 [b]	41.56 ± 0.02 [b]	7.09 ± 0.11 [a]	41.89 ± 0.15 [b]
C18:2	37.37 ± 0.48 [b]	37.50 ± 0.47 [b]	37.53 ± 0.11 [b]	1.53 ± 0.04 [a]	37.37 ± 0.48 [b]
C18:3	1.08 ± 0.01 [a]	1.11 ± 0.11 [a]	1.30 ± 0.01	-	1.08 ± 0.01 [a]
C20:0	0.25 ± 0.00 [a]	0.15 ± 0.05 [a]	0.18 ± 0.01 [a]	-	0.25 ± 0.00 [a]
SFA	18.83 ± 0.34 [a]	18.82 ± 0.10 [a]	18.98 ± 0.02 [a]	91.43 ± 0.08 [b]	18.83 ± 0.34 [a]
PUFA	38.44 ± 0.66 [b]	38.60 ± 0.47 [b]	38.83 ± 0.13 [b]	1.53 ± 0.04 [a]	38.44 ± 0.66 [b]
MUFA	42.03 ± 0.21 [b]	42.07 ± 0.07 [b]	41.67 ± 0.03 [b]	7.09 ± 0.11[a]	42.03 ± 0.21 [b]
UFA	80.47 ± 0.45[b]	80.67 ± 1.02 [b]	80.50 ± 0.10 [b]	8.62 ± 0.32 [a]	80.47 ± 0.45[b]
ECN(%)					
28	-	-	-	-	-
30	-	-	-	1.97 ± 0.15 [a]	-
32	-	-	-	12.37 ± 0.13 [a]	-
34	-	-	-	16.99 ± 0.14 [a]	-
36	-	-	-	20.27 ± 0.88 [a]	-
38	-	-	-	17.33 ± 0.28 [a]	-
40	-	-	-	11.59 ± 0.21 [a]	-
42	2.71 ± 0.04 [a]	2.78 ± 0.07 [a]	3.06 ± 0.06 [a]	7.21 ± 0.18 [b]	2.71 ± 0.04 [a]
44	29.55 ± 0.04 [a]	28.38 ± 0.06 [a]	29.08 ± 0.55 [a]	2.44 ± 0.13 [b]	29.55 ± 0.04 [a]
46	47.03 ± 1.00 [a]	47.19 ± 0.31 [a]	46.70 ± 0.04 [a]	3.47 ± 0.09 [b]	47.03 ± 1.00 [a]
48	20.71 ± 0.92 [a]	21.66 ± 0.30 [a]	21.16 ± 0.58 [a]	1.40 ± 0.12 [b]	20.71 ± 0.92 [a]
50	-	-	-	0.57 ± 0.09 [a]	-
52	-	-	-	0.52 ± 0.02 [a]	-

* *SFA* saturated fatty acids, *PUFA* polyunsaturated fatty acids, *MUFA* monounsaturated fatty acids, *UFA* unsaturated fatty acids, "-" not detected. The equivalent carbon number (ECN) is equal to the total number of carbon atoms in the fatty acid minus twice the number of double bonds. The data in the table is the mean ± standard deviation, "-" represents no detectable; the different letters in the peer represent a significant difference (p<0.05).

Table 2 Minor constituent composition of five vegetable oils.

	RBO-D	RBO-P	RBO-S	CNO-C	CNO-J
Oryzanol (mg/kg oil)					
	1543 ± 26 [a]	5605 ± 137 [b]	25611 ± 2571 [c]	-	-
Tocopherols (mg/kg oil)					
α- Tocopherol	60 ± 2 [a]	245 ± 13 [c]	214 ± 1 [b]	3 ± 0 [d]	3 ± 0 [d]
β- Tocopherol	-	-	-	-	-
γ- Tocopherol	-	-	-	-	-
δ - Tocopherol	-	-	-	-	-
Total tocopherol	60 ± 2 [a]	245 ± 13 [c]	214 ± 1 [b]	3 ± 0 [d]	3 ± 0 [d]
Phytosterols (mg/kg oil)					
Δ^5- Campesterol	701 ± 4 [a]	1114 ± 51 [b]	1488 ± 51 [c]	25 ±1 [d]	24 ± 1 [d]
Stigmasterol	499 ± 21 [a]	793 ± 33 [b]	873 ± 46[c]	43 ± 3 [d]	38 ± 0 [d]
β- sitosterol	2664 ± 94 [a]	3669 ± 165 [b]	3933 ± 167 [c]	118 ± 4 [d]	124 ± 1 [d]
Fucosterol	302 ± 12 [b]	240 ± 9 [a]	383 ± 54[c]	40 ± 2 [d]	42 ± 1 [d]
Total sterol	4167 ± 249 [a]	5819 ± 258 [b]	6677 ± 315 [c]	225 ± 10 [d]	228 ± 4 [d]
Polyphenols (mg GAE/kg oil)					
	21 ± 1 [a]	60 ± 2 [b]	198 ± 7 [c]	4 ± 0 [d]	-

* The data in the table is the mean ± standard deviation, "-" represents no detectable; the different letters in the peer represent a significant difference (p<0.05).

Table 3 EC_{50} values for the inhibition of peroxyl radical-induced DCFH oxidation by five vegetable oil emulsions (mean ± SD, n=3) and their cytotoxic concentrations.

	EC_{50} [A]	CV (%)	Cytotoxicity [B]	CAA (μmol of QE/100 mg oil)
Quercetin	4.9 ± 0.1	2.0	32	
RBO-D	5.9 ± 0.2 [b]	3.4	>40	83.7 ± 0.4 [b]
RBO-P	4.6 ± 0.3 [c]	6.5	50	106.1 ± 0.4 [c]
RBO-S	0.6 ± 0.1 [d]	16.7	20	846.6 ± 0.6 [d]
CNO-C	4.6 ± 0.3 [c]	6.5	>20	107.2 ± 0.2 [c]
CNO-J	6.4 ± 0.2 [a]	3.1	>40	76.7 ± 0.2 [a]

[A] The EC_{50} value of quercetin is expressed as μmol and the EC_{50} value of RBO-D, RBO-P, RBO-S, CNO-C, and CNO-J emulsions are expressed as mg/mL.

[B] The cytotoxicity value of quercetin is expressed as μmol and the cytotoxicity value of RBO-D, RBO-P, RBO-S, CNO-C, and CNO-J emulsions are expressed as mg/mL. QE is quercetin equivalents.

*Different lowercase letters indicate significant differences (p<0.05).

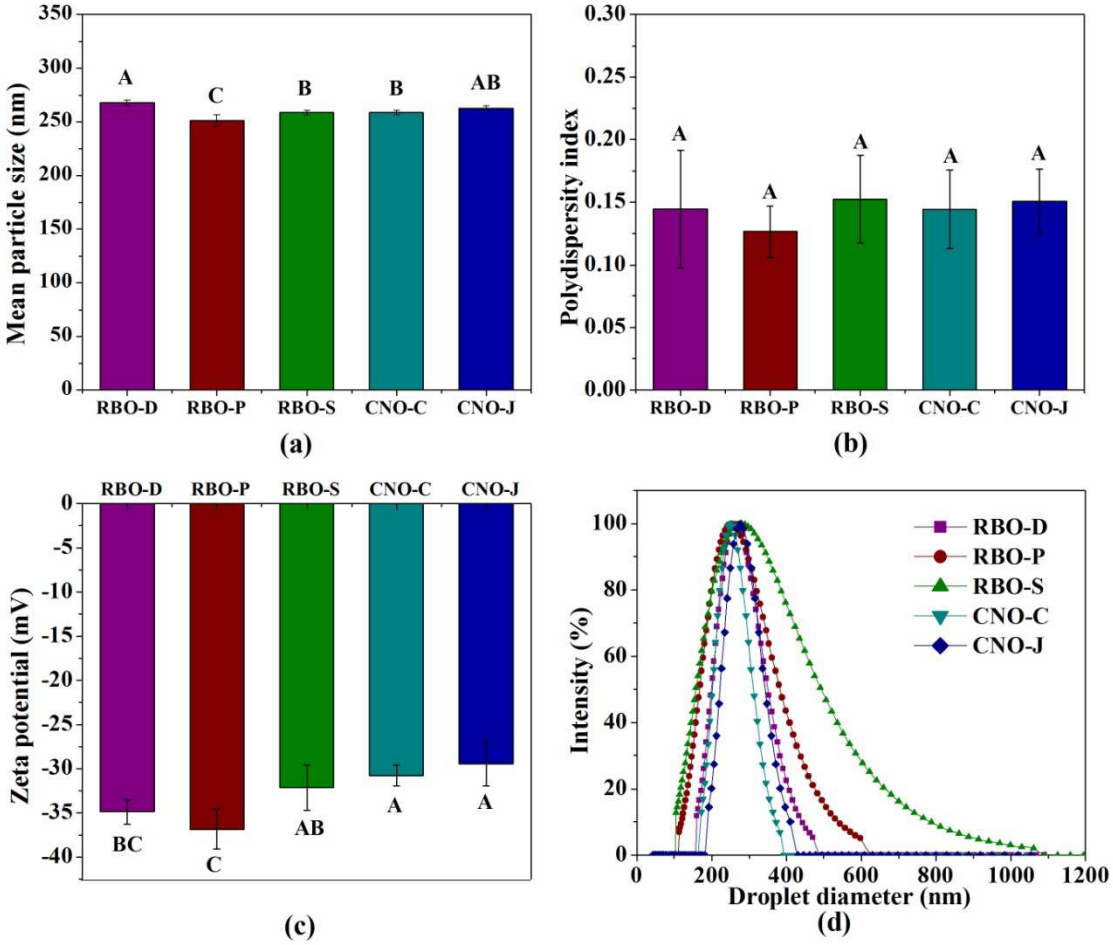

Fig. 1. (a) Mean particle size, (b) PDI and (c) zeta potential (d) droplet size distribution of RBO-D, RBO-P, RBO-S, CNO-C, and CNO-J emulsions obtained using high homogenizing pressures (60 MPa, 3 cycle times). *Different letters indicate significant differences (p<0.05) of the mean particle size, PDI or zeta potential between five oil emulsions.

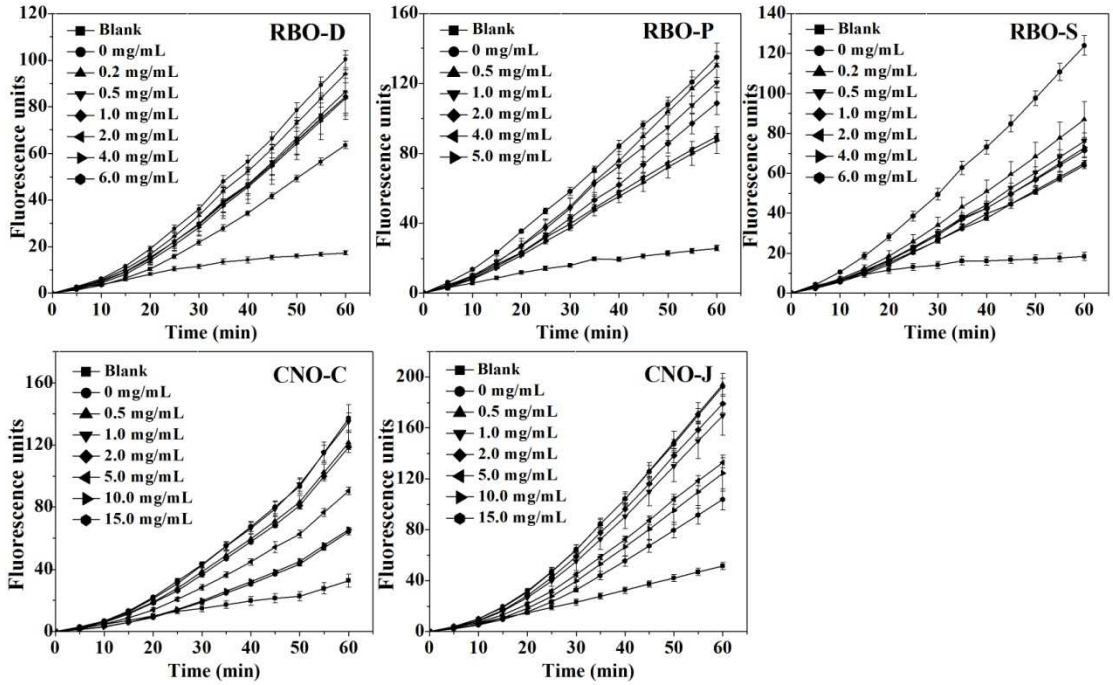

Fig. 2. Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by RBO-D, RBO-P, RBO-S, CNO-C, and CNO-J emulsions over time. The curves shown in each graph are from a single experiment (mean ± SD, n = 3).

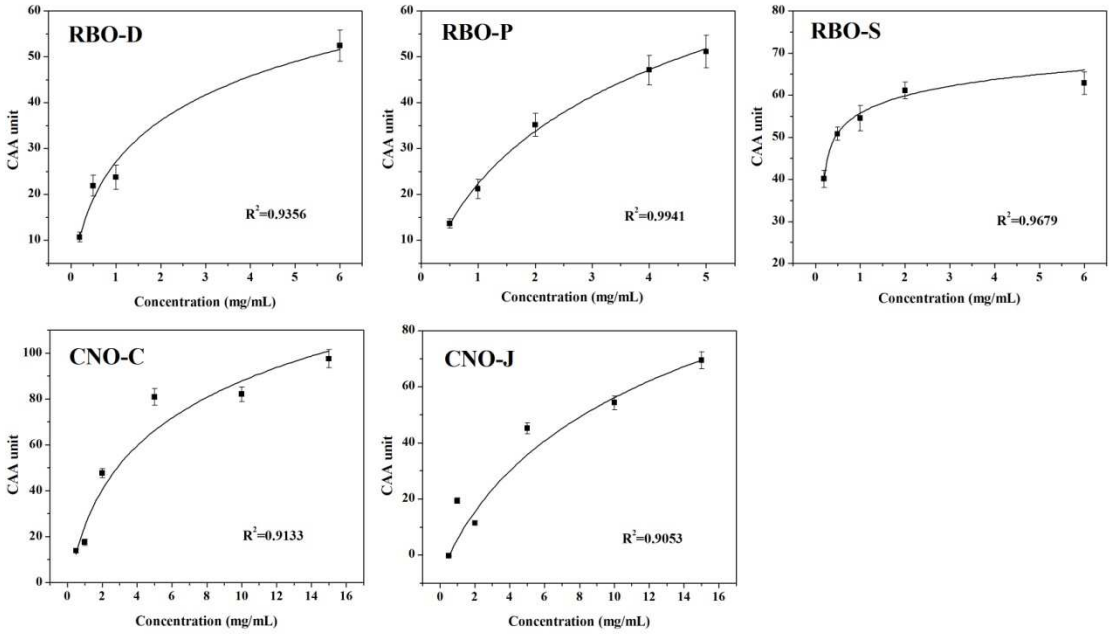

Fig. 3. Dose-response curves for inhibition of peroxyl radical-induced DCFH oxidation by RBO-D, RBO-P, RBO-S, CNO-C, and CNO-J emulsions. The curves shown are each from a single experiment (mean ± SD, n = 3).

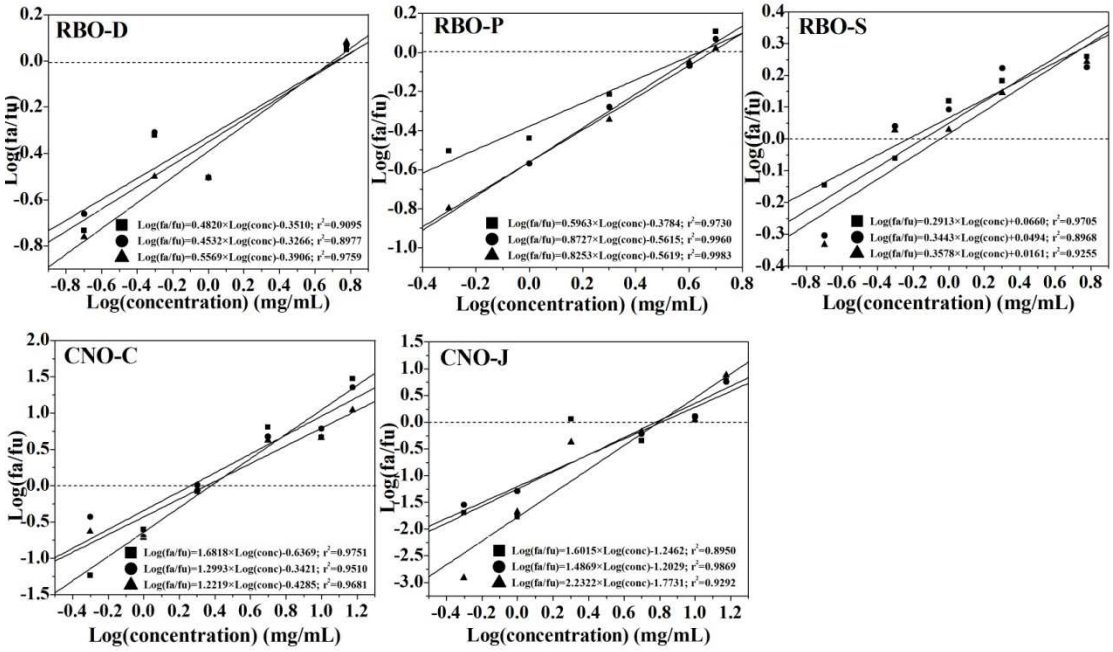

Fig. 4. Median effect plots for inhibition of peroxyl radical-induced DCFH oxidation by RBO-D, RBO-P, RBO-S, CNO-C, and CNO-J emulsions. The curves shown are from a single experiment (mean ± SD, n = 3).

Highlights:

- Emulsions rich in long-chain unsaturated triglycerides are more dispersed in size distribution.
- Oil with medium-chain saturated triglyceride is easier to result in strong CAA in oil emulsions.
- The result of CAA was the combined effects of triglycerides and micronutrients of the vegetable oil

Declarations of interest: none.