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Antioxidant activity of resveratrol ester derivatives in food and biological model systems

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ABSTRACT

Resveratrol (R) was lipophilized by esterification in order to facilitate its application in a wide range of products and to possibly enhance its bioactivity. Twelve resveratrol derivatives were prepared using acyl chlorides of different chain length (C3:0–C22:6) and their antioxidant activities assessed. While resveratrol showed the highest antioxidant activity in oil-in-water emulsion, its derivatives (RC6:0, RC8:0, RC10:0, RC12:0, RC16:0) showed better antioxidant activity in a bulk oil system. Resveratrol esters RC20:5n-3 (REPA) and RC22:6n-3 (RDHA) showed the highest antioxidant activity when added to ground meat. Meanwhile, resveratrol derivatives (RC3:0–RC14:0) had better hydrogen peroxide scavenging activity than resveratrol. All test compounds except resveratrol and REPA inhibited copper-induced LDL oxidation. Moreover, test compounds effectively inhibited hydroxyl radical induced DNA scission. These results suggest that resveratrol derivatives could potentially serve as functional food ingredients and supplements for health promotion and disease risk reduction.

1. Introduction

Lipid oxidation is a major deteriorative process in food. It is responsible for off-flavour development, decreasing of nutritional value, and generation of potentially toxic compounds (Frankel, 1980). The most common lipid oxidation process encountered in food is autoxidation in which the free radicals formed lead to further adverse effects once consumed (Choe & Min, 2006). Concerns about lipid oxidation are not limited to food, because the free radicals formed can also attack different cells in the body, thus causing aging and a myriad of diseases such as cancer, cardiovascular ailments and immune system deficiencies (Percival, 1998). Antioxidants are generally phenolic compounds that can interfere with free radical chain reaction by donating a hydrogen atom or an electron to free radicals (Frankel, 1980). By retarding oxidation, they can also prevent certain diseases related to oxidative stress such as skin lesions, atherosclerosis, pulmonary dysfunction, cancer, and inflammatory disorders, among others (Percival, 1998).

Resveratrol is a phenolic stilbenoid compound with powerful antioxidant activity. It also has preventive effects on inflammation, cardiovascular disease and cancer (Jang et al., 1997; Wang, et al., 2002; Donnelly et al., 2004). However, some studies have failed to reflect these beneficial effects of resveratrol, possibly due to its high absorption but low bioavailability (Bove, Lincoln, & Tsan, 2002; Walle, Hsieh, Delegge, Oatis, & Walle, 2004). In addition, like some other phenolic compounds, application of resveratrol could be compromised due to its hydrophilicity when used in lipophilic systems (Zhong & Shahidi, 2011). However, this problem could be overcome by structural modification. In fact, there are several studies reporting maximizing antioxidant activities via structure modification, such as those for chlorogenic acid (Laguerre et al., 2009), epigallocatechin gallate (EGCG) (Zhong & Shahidi, 2011), and rosmarinic acid (Panya et al., 2012), among others. Moreover, EGCG esterified with polyunsaturated fatty acids (PUFA), especially docosahexaenoic acid (DHA), provided a novel bioactive product that was a composite of the two bioactive compounds since DHA itself possesses health benefits (Zhong & Shahidi, 2012). For this reason, resveratrol was esterified with 12 different acyl chlorides (propionyl chloride, butyryl chloride, caproyl chloride, capryloyl chloride, capryl chloride, lauroyl chloride, myristoyl chloride, palmitoyl chloride, stearoyl chloride, oleoyl chloride, eicosapentaenoyl chloride, and docosahexaenoyl chloride) as described elsewhere (Oh & Shahidi, 2017). The esterification process is expected to improve their use in lipophilic systems such as lipid-based food and biological environments.

According to Vlachogianni, Fragopoulou, Kostakis, and Antonopoulou (2015), 4′-acetylated resveratrol showed a better DPPH radical scavenging activity than its 3-acetylated isomer (Resveratrol [R] > 4′-acetylated resveratrol > 3-acetylated resveratrol > 3,5-diacetylated resveratrol). However, in the non-enzymatic linoleic peroxidation assay, they reported that 3-acetylated resveratrol exhibited a

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better antioxidant activity than 4′-acetylated resveratrol (3,5-diacetylated resveratrol > 3-acetylated resveratrol > R > 4′-acetylated resveratrol > 3,4′-diacetylated resveratrol). Moreover, they reported that 3,5-diacetyl-R exhibited the most powerful effect in non-enzymatic linoleic acid peroxidation even though it had lost two of its hydroxyl groups. The effect of esterification position and number of esterification substitution is still unknown. Since the resultant esters still have antioxidant activity, it is of interest to see whether the mixture of mono- and diesters show an additive, synergistic, or antagonistic effect.

In the case of oral administration of ester compounds, the ester bond might undergo hydrolysis. Biasutto, Marotta, Marchi, Zoratti, and Paradisi (2007) reported that some quercetin esters underwent partial hydrolysis, when they passed through a monolayer of MDCK-1, MDCK-2, and Caco-2 cells. Hydrolysis of ester was dependent on the compounds examined. According to Pokorski, Marczak, Dymecka, and Suchocki (2003) ascorbyl palmitate treated cats showed more recovery of ascorbate in their brain tissues compared to ascorbic acid treated cats. Therefore, it might be hypothesized that resveratrol esters would show beneficial effect even if they underwent hydrolysis.

In this work, selected food and biological model systems were used to fill the existing knowledge gap in the literature on antioxidant potential of resveratrol derivatives in food and for potential health promotion and disease risk reduction. Potential synergistic effects of resveratrol with PUFA were also examined in this contribution.

2. Materials and methods

2.1. Materials

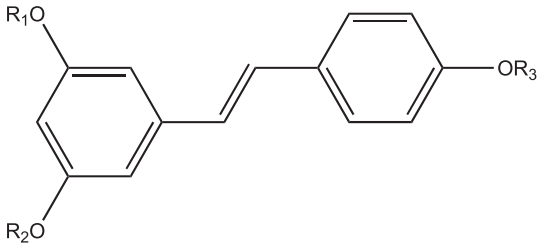
Resveratrol and DHA single cell oil (DHASCO) were obtained from DSM Nutritional Products (Columbia, MD, USA). Eicosapentaenoic acid (EPA) ethyl ester, from Mochida Pharmaceutical CO., LTD. (Tokyo, Japan), was provided by Professor Kazuo Miyashita. Preparation of EPA and DHA was described by Zhong and Shahidi (2011). All Chemicals were purchased from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada) or Fisher Scientific Ltd (Ottawa, ON, Canada) and were used without any further purification. All solvents used were of ACS or HPLC grade.

2.2. Preparation of resveratrol derivatives

Resveratrol derivatives were prepared as described by Oh and Shahidi (2017). Briefly, resveratrol was esterified with acyl chlorides of propionic acid (C3:0), butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), eicosapentaenoic acid (C20:5, EPA), and docosahexaenoic acid (C22:6, DHA). The chemical structures of resveratrol derivatives, after HPLC separation, were determined by MS and different types of NMR. The mono- and diesters of crude products (Fig. 1) were used to assess their antioxidant potential in selected food and biological model systems.

2.3. Antioxidant activity in a meat model system (thiobarbituric acid reactive substances, TBARS)

A ground meat model system was used to determine antioxidant activity of resveratrol and its derivatives (Wettasinghe & Shahidi, 1999) with modification. Forty grams of ground pork were mixed with 10 g of deionized water in Mason jars. One millilitre of test compounds (1mM) or a positive control (butylated hydroxytoluene, BHT, 1 mM) was added the meat. A blank with no antioxidant was also prepared. The meat samples were mixed and cooked for 40 min in a water bath at 80 °C with intermittent stirring. The contents were transferred into plastic bags and stored for 7 days at 4 °C. Meat samples were analyzed on days 0, 3, 5, and 7 for their oxidative state using the TBARS test as described by Shahidi and Hong (1991) with slight modification. Trichloroacetic acid (10%, w/v, 2.5 mL) was added to meat samples (1g) in centrifuge tubes



Resveratrol: R_1=R_2=R_3=H
Monoesters: R_1=X, R_2=R_3=H and R_1=R_2=H, R_3=X
Diesters: R_1=R_2=X, R_3=H and R_1=R_3=X, R_2=H
X : C_3H_5O (C3:0)
C_4H_7O (C4:0)
$C_6H_{11}O$ (C6:0)
$C_8H_{15}O$ (C8:0)
$C_{10}H_{19}O$ (C10:0)
$C_{12}H_{23}O$ (C12:0)
$C_{14}H_{27}O$ (C14:0)
$C_{16}H_{31}O$ (C16:0)
$C_{18}H_{35}O$ (C18:0)
$C_{18}H_{33}O$ (C18:1)
$C_{20}H_{29}O$ (C20:5, EPA)
$C_{22}H_{31}O$ (C22:6, DHA)

Fig. 1. Structures of resveratrol derivatives.

which were then vortexed for 2 min. Subsequently, TBA reagent (0.02 M, 2.5 mL) was added and vortexed again for 30 s. After centrifugation at 3000*g* for 10 min, the supernatants were filtered through a Whatman No.3 filter paper. The samples were kept in a water bath at 95 °C for 45 min, then cooled to room temperature. A precursor of malondialdehyde (MDA), 1,1,3,3-tetramethoxypropane, was used for constructing a standard curve. The absorbance of pink TBA-MDA adducts was read at 532 nm. TBARS values were calculated using the standard curve and expressed as milligrams of MDA equivalents per kilogram of sample.

2.4. Antioxidant activity of resveratrol derivatives in bulk oil

Stripped corn oil was prepared according to a simplified stripping method. The oil (100 g) with hexane (1 L) was subjected to stripping using activated silicic acid (150 g) and charcoal (25 g). The eluting solvent was then removed using a rotary evaporator at 40 °C.

The stripped oil (1 g) was weighed into 10 mL clear glass vials and loosely capped in order to induce oil oxidation. Resveratrol and its derivatives (75 μM, 100 μL) in ethanol were added, and the solvent was then removed under a stream of nitrogen. The vials were wrapped in aluminum foil to protect samples from light and stored in a forced air oven (Precision Scientific Co., Chicago, IL, USA) at 60 ± 0.5 °C. Samples were withdrawn on day 0, 1, 3, and 6 for analyses. Conjugated dienes and *p*-anisidine values were used for monitoring oxidative products according to the , AOAC (Conjugated dienes, 1980; *p*-anisidine values, 1990) methods.

2.5. Antioxidant activity of resveratrol derivatives in oil-in-water emulsion (β-carotene bleaching assay)

Beta-carotene bleaching assay was used to determine antioxidant activity of resveratrol and its derivatives in an oil-in-water emulsion according to Zhong and Shahidi (2012). An aliquot (1.2 mL) of β-

carotene (10 mg) dissolved in 10 mL of chloroform was transferred into a flask containing linoleic acid (40 mg) and Tween 40 (400 mg). A blank was also prepared (40 mg of linoleic acid + 400 mg of Tween 40 without β-carotene). Chloroform was removed under a stream of nitrogen and then 100 mL of oxygenated distilled water were added and stirred vigorously. Resveratrol, its derivatives (0.5 mL, 1 mM in 100% ethanol) and the control (0.5 mL, 100% ethanol) were mixed with aliquots (4.5 mL) of the above emulsion. Each sample blank (40 mg of linoleic acid + 400 mg of Tween 40 + 0.5 mg of samples without β-carotene) was also prepared. The absorbance of the tubes was read immediately at 470 nm and then incubated in a shaking water bath at 50 °C. The absorbance was read over 105 min period at 15 min intervals. Antioxidant activity of resveratrol and its derivatives in oil-in-water emulsion was subsequently calculated using the following equation.

$$\text{Antioxidant activity (\%)} = [1-(S_0-S_t)/(C_0-C_t)]*100$$

where S_0 and S_t are corrected absorbance values for test compounds measured at zero time and after incubation, respectively; while C_0 and C_t are corrected absorbance values for the control at zero time and after incubation, respectively.

2.6. Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging activity of resveratrol and its derivatives was determined according to the method explained by Chandrasekara and Shahidi (2011) with slight modification. The standard (ascorbic acid) and test compounds dissolved in 100% ethanol (1 mM, 0.4 mL) were added to 0.6 mL of 40 mM H_2O_2 (in 45 mM sodium phosphate buffer solution, pH 7.4) and 1 mL of buffer solution. The absorbance was read at 230 nm after 40 min of incubation at 30 °C. Blanks were run for each sample; buffer solution was added instead of H_2O_2. A standard curve was prepared with ascorbic acid (AA). The results were expressed as micromoles of AA equivalents per micromoles of each compound.

2.7. Inhibition against copper-induced low-density lipoprotein (LDL) oxidation

The inhibition of LDL oxidation induced by copper was determined according to Zhong and Shahidi (2012). Human LDL was dialyzed in 10 mM phosphate buffer (pH 7.4, 0.15 M NaCl) using a dialysis tube (molecular weight cutoff of 12–14 kDa; Fisher Scientific, Nepean, ON, Canada) at 4 °C under a nitrogen blanket in the dark for 12 h. Diluted LDL (0.04 mg LDL/mL, 0.8 mL) was mixed with resveratrol and its derivatives (5 μM, 100 μL). The samples and cupric sulphate ($CuSO_4$) were pre-incubated at 37 °C for 15 min. The reaction was initiated by adding $CuSO_4$ (50 μM, 100 μL) and measured immediately at 234 nm. The samples were then incubated at 37 °C for 8 h. The formation of conjugated dienes (CD) was monitored at 234 nm using a diode array spectrophotometer (Agilent, Palo Alto, CA, USA). The blanks were run for each sample by replacing LDL and $CuSO_4$ with phosphate buffer for background correction.

2.8. Inhibition against hydroxyl radical-induced DNA scission

Inhibitory activity of resveratrol and its derivatives against DNA scission induced by hydroxyl radical was determined according to the method described by Ambigaipalan and Shahidi (2015) with slight modification. Supercoiled pBR 322 DNA (50 μg/mL) was dissolved in 10 mM phosphate buffer (pH 7.4), and resveratrol and its derivatives dissolved in ethanol were diluted with phosphate buffer. In an eppendorf tube, test compound (12.5 μM, 2 μL), phosphate buffer (2 μL), DNA (50 μg/mL, 2 μL), $FeSO_4$ (0.5 mM, 2 μL), and H_2O_2 (1 mM, 2 μL) were added. A control (DNA with radicals) and a blank (DNA only) were also prepared. After incubation at 37 °C for 1 h, 1 μL of the loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, and 50% glycerol) was added. The mixture was loaded onto 0.7% agarose gel (50 mL). The gel was prepared in Tris–acetic acid–EDTA (ethylenediaminetetraacetic acid) buffer (TAE, 40 mM, pH 8.5) with SYBR safe gel stain (5 μL). Electrophoresis was performed at 80 V for 90 min in TAE buffer using a horizontal mini gel electrophoresis system (Owl Separation Systems Inc., Portsmouth, NH, USA) with a 300 V power supply (VWR International Inc., West Chester, PA, USA). The DNA bands were visualized under UV light using the Alpha-Imager gel documentation system (Cell Biosciences, Santa Clara, CA, USA) and the data processing was performed with the Chemi-Imager 4400 software (Cell Biosciences). The retention of supercoiled DNA strand (%) was calculated according to the following equation:

$$\text{DNA retention (\%)} = (\text{DNA}_{\text{sample}}/\text{DNA}_{\text{blank}})*100$$

2.9. Statistical analysis

Statistical analysis was performed Tukey's HSD test with one way ANOVA ($p < 0.05$) using IBM statistics version 21 (IBM Corp., Armonk, NY, USA).

3. Results and discussion

3.1. Antioxidant activity in a meat model system (TBARS)

The thiobarbituric acid (TBA) method was employed to assess the effect of resveratrol and its derivatives in the control of oxidation in a meat model system. The secondary oxidative products, mainly aldehydes and ketones, are responsible for off-flavour development and loss of nutrients in muscle foods (Cesa, 2004). MDA, one of the degradation products of polyunsaturated fatty acids, reacts with TBA. The MDA-TBA so produced has a pink colour, which absorbs at 530–535 nm as well as some other aldehydes and dialdehydes, therefore TBARS are usually reported as MDA equivalents (Shahidi & Zhong, 2015). In this study, BHT was employed as a positive control. As shown in Table 1, all samples showed an increasing trend of TBARS during cold storage (4 °C). The ground pork system containing resveratrol with unsaturated fatty acids (C18:1, EPA, DHA) had the lowest TBARS values at the

Table 1
TBARS values of cooked ground pork model system over a 7-day period in presence of resveratrol and its derivatives (MDA eq./kg).

Sample	Storage period			
	Day 0	Day 3	Day 5	Day 7
Control	1.03 ± 0.00^{b}	2.25 ± 0.05^{ab}	2.84 ± 0.09^{ab}	2.94 ± 0.09^{a}
BHT	1.19 ± 0.06^{a}	2.10 ± 0.04^{bc}	2.74 ± 0.04^{b}	2.74 ± 0.04^{b}
R	0.86 ± 0.01^{c}	1.56 ± 0.02^{h}	1.86 ± 0.07^{fg}	2.35 ± 0.05^{cde}
RC3:0	0.46 ± 0.01^{fg}	1.25 ± 0.06^{j}	1.89 ± 0.05^{fg}	2.19 ± 0.03^{ef}
RC4:0	0.62 ± 0.01^{de}	1.60 ± 0.04^{h}	2.12 ± 0.03^{e}	2.27 ± 0.05^{cde}
RC6:0	0.68 ± 0.01^{d}	1.35 ± 0.03^{ij}	1.78 ± 0.03^{g}	2.24 ± 0.06^{def}
RC8:0	0.89 ± 0.03^{c}	1.76 ± 0.02^{fg}	2.34 ± 0.03^{d}	2.74 ± 0.09^{b}
RC10:0	0.83 ± 0.05^{c}	1.62 ± 0.04^{gh}	2.10 ± 0.05^{e}	2.81 ± 0.04^{ab}
RC12:0	1.04 ± 0.01^{b}	1.95 ± 0.03^{cde}	2.41 ± 0.10^{cd}	2.80 ± 0.03^{ab}
RC14:0	0.86 ± 0.02^{c}	2.26 ± 0.05^{a}	2.99 ± 0.7^{a}	2.70 ± 0.06^{b}
RC16:0	1.01 ± 0.04^{b}	2.26 ± 0.03^{cd}	2.99 ± 0.08^{c}	2.70 ± 0.02^{b}
RC18:0	0.53 ± 0.03^{ef}	1.94 ± 0.06^{de}	2.47 ± 0.07^{cd}	2.39 ± 0.08^{cd}
RC18:1	0.36 ± 0.03^{h}	1.47 ± 0.08^{hi}	2.03 ± 0.03^{ef}	2.44 ± 0.07^{c}
REPA	0.42 ± 0.00^{gh}	1.79 ± 0.02^{ef}	1.71 ± 0.03^{g}	2.00 ± 0.06^{g}
RDHA	0.42 ± 0.04^{gh}	1.56 ± 0.12^{h}	1.82 ± 0.05^{g}	2.08 ± 0.05^{fg}

Each value was replicated three times. Values in the same column with different letters were significantly different at $p < 0.05$ performed by Tukey's HSD test. Resveratrol (R) or its derivatives (RC3:0, RC4:0, RC6:0, RC8:0, RC10:0, RC12:0, RC14:0, RC16:0, RC18:0, RC18:1, REPA and RDHA) are resveratrol esters of propionic acid, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively.

beginning of the storage after cooking (day 0). From day 3 to day 7, most of the test compounds except RC14:0 displayed lower TBARS values compared to the control. RC3:0 and RC6:0 had the lowest TBARS value among all tested compounds after 3 days of storage, significantly lower than that of resveratrol itself ($p < 0.05$). Meanwhile RC3:0, RC6:0, REPA, and RDHA showed some, but insignificant difference with resveratrol after 5 days of storage (in order RC3:0 > R > RDHA > RC6:0 > REPA, $p > 0.05$). However, REPA and RDHA were able to inhibit the production of TBARS significantly compared to resveratrol after 7 days of storage ($p < 0.05$). According to Zhong and Shahidi (2012), inhibition of β-carotene bleaching by EGCG and its derivatives was correlated with the inhibition of TBARS production and their lipophilicity (EGCG-C18:0 > EGCG-DHA > EGCG-EPA > EGCG). However, no correlation existed between TBARS value and lipophilicity in this study. There was a possibility that the ester bonds could be hydrolyzed during cooking (80 °C), which might lead to insignificant difference with resveratrol itself. Moreover, REPA and RDHA had PUFA, are able to produce aldehydes that could increase TBARS. However, REPA and RDHA showed the lowest TBARS values among all tested compounds after 7 days of storage ($p < 0.05$). Moreover, most of the test compounds, except resveratrol with medium chain fatty acids, exhibited a better inhibition of the formation of TBARS when compared with BHT during 7 days of storage. The amount of BHT used was low (equivalent to 5 ppm) compared to other similar studies (Ambigaipalan & Shahidi, 2015), therefore the positive control might show lower antioxidant activity than other studies. However, some of the resveratrol esters were able to inhibit TBARS formation and this was stronger statistically than the positive control at the same concentration. Therefore, the results so obtained suggest that resveratrol derivatives might be used as potential antioxidants in cooked muscle foods in order to provide better oxidative stability to the products.

3.2. Antioxidant activity in bulk oil

Corn oil used in this study was stripped of its endogenous antioxidants in order to avoid their contribution to oxidative stability of the oil containing the test compounds. Major fatty acids of corn oil are oleic acid (C18:1, 34.1%) and linoleic acid (C18:2, 47.9%) (Ferrari, Schulte, Esteves, Brühl, & Mukherjee, 1996). Generally, oxidation is initiated by the abstraction of a hydrogen atom attached to allylic or bis-allylic carbon atom (carbon next to the double bond) due to their low dissociation energy (Shahidi & Zhong, 2009). After losing its hydrogen atom, the alkyl radical formed is stabilized by resonance delocalization, along with possible formation of trans isomers as well as conjugated dienes (polyunsaturated fatty acids) (Choe & Min, 2006). The conjugated diene content was measured at 233 nm. Conjugated dienes could, however, be broken to secondary oxidation products, such as aldehydes, ketones, alcohols and hydrocarbons (Choe & Min, 2006). For secondary oxidation products, *p*-anisidine value was measured at 350 nm. Conjugated diene formation of corn oil in this study is shown in Table 2. The oils with test compounds exhibited a similar lag phase of 3 days. On day 6, most of the test compounds were able to retard the formation of conjugated dienes significantly except RC3:0 and RDHA ($p < 0.05$). Although resveratrol derivatives lost their hydroxyl groups, some derivatives (RC6:0, RC8:0, RC10:0, RC12:0, RC16:0) showed significantly better antioxidant activity than resveratrol itself ($p < 0.05$). This result also contradicts polar paradox, as polar antioxidants generally are more efficacious than nonpolar antioxidants in bulk oil systems (more discussion in 3.3). In addition, we hypothesized that resveratrol with polyunsaturated fatty acids (PUFA), namely EPA and DHA might produce the same or even a higher amount of conjugated dienes than the control because PUFA connected to resveratrol could also be oxidized and form conjugated dienes. However, REPA inhibited the oxidation to nearly the same extent as resveratrol itself (no significant difference). RC3:0 had very little effect on antioxidant activity but the antioxidant activity of other resveratrol derivatives from RC4:0 to RC8:0 increased as the lipophilicity increased. However, the antioxidant activity decreased after the antioxidant activity reached a maximum point (RC8:0). This has been observed in several studies; this phenomenon was coined as cutoff effect or nonlinear theory (Laguerre et al., 2009; Panya et al., 2012; Laguerre et al., 2015). Laguerre et al. (2009) studied the antioxidant activities of chlorogenic acid and its alkyl esters in emulsions to examine the effect of chain length on antioxidant activity. They observed a drastic decrease in antioxidant activity after dodecyl chain (C12:0). Furthermore, Panya et al. (2012) observed a nonlinear phenomenon in DPPH radical scavenging assay using rosmarinic acid and its alkyl esters.

The *p*-anisidine value of the oils with test compounds (RC6:0, RC8:0, RC10:0, RC12:0, RC16:0, RC18:0) on day 6 showed a significantly better antioxidant activity than the parent molecule ($p < 0.05$, Table 2). RC8:0 showed the highest antioxidant activity among all compounds tested while RC3:0 and RDHA had no effect compared to the control. This result correlated well with conjugated dienes and their correlation coefficient was 0.96.

3.3. Antioxidant activity of resveratrol derivatives in oil-in-water emulsion (β-carotene bleaching assay)

A β-carotene bleaching assay was used to evaluate the antioxidant activity of resveratrol and its derivatives in an oil-in-water emulsion. When two immiscible liquids exist together, one liquid can form a dispersion of small droplets in the other liquid, known as emulsion (McClements, 2002). Tween 40 and linoleic acid, used in this assay, are amphiphilic with a hydrophilic head and a lipophilic tail, thus can form small spherical micelle in the water (McClements, 2002). Meanwhile, β-carotene, a lipophilic compound, stays inside the micelle. The heat induces oxidation, and linoleic acid forms hydroperoxides and free radicals as a result of losing a hydrogen atom. Beta-carotene is attacked from those oxidative products, therefore it loses its yellow-orange colour. (Ambigaipalan & Shahidi, 2015). The change of colour could be monitored spectrometrically at 470 nm. Table 3 indicates that resveratrol and its derivatives inhibited the decolourization of β-carotene by 11.4–71.2% over the 105 min incubation period. Resveratrol had the highest inhibition among all compounds tested. Among derivatives, resveratrol with short chain fatty acids (RC3:0 and RC4:0) showed better antioxidant activity than other derivatives. The antioxidant activity of resveratrol derivatives in oil-in-water emulsion was well correlated with ABTS radical cation scavenging activity with a correlation coefficient of 0.84 (Oh & Shahidi, 2017). The antioxidant activity of resveratrol derivatives in oil-in-water system did not correspond with polar paradox. According to polar paradox, nonpolar antioxidants tend to be more effective than polar ones in oil-in-water emulsions. This is because nonpolar antioxidants may stay around the lipid droplet where oxidation occurs (Laguerre et al., 2015). However, several studies have demonstrated that not all antioxidants follow the trend that can be explained by polar paradox (Torres de Pinedo, Peñalver, & Morales, 2007; Stöckmann, Schwarz, & Huynh-Ba, 2000). Torres de Pinedo et al. (2007) studied antioxidant activity of phenolic compounds containing alcohols with different alkyl chain lengths such as protocatechuic alcohol (C1), hydroxytyrosol (C2), and dihydrocaffeoyl alcohol (C3). They found an increase of antioxidant activity in bulk oil (C3 > C2 > C1), which contradicts the polar paradox theory. Stöckmann et al. (2000) observed different trends of antioxidant activity using various emulsifiers in oil-in-water system and suggested that the application of polar paradox might need to be narrowed down to the emulsions containing phospholipids as emulsifiers.

3.4. Hydrogen peroxide (H_2O_2) scavenging activity

Superoxide dismutase in the human body can generate H_2O_2 in order to remove superoxide radical ($O_2^{\cdot -}$) (Halliwell, 1991). In addition, activated phagocyte can produce H_2O_2 to protect the body from

Table 2
Conjugated dienes formation and *p*-anisidine values of stripped corn oil over a 6-day period in the presence of resveratrol and its derivatives.

Sample	Conjugated dienes formation				*p*-Anisidine values			
	Storage period				Storage period			
	Day 0	Day 1	Day 3	Day 6	Day 0	Day 1	Day 3	Day 6
Control	0.21 ± 0.02^{e}	0.50 ± 0.01^{bcd}	0.62 ± 0.01^{ab}	5.46 ± 0.03^{a}	22.36 ± 9.10^{a}	11.80 ± 0.21^{cde}	17.66 ± 0.44^{bcd}	164.99 ± 0.76^{a}
R	0.22 ± 0.01^{e}	0.48 ± 0.02^{de}	0.57 ± 0.00^{d}	4.42 ± 0.08^{ef}	16.93 ± 6.51^{ab}	12.02 ± 0.31^{cd}	16.48 ± 0.26^{d}	84.82 ± 0.34^{e}
RC3:0	0.24 ± 0.00^{e}	0.48 ± 0.02^{cde}	0.63 ± 0.00^{a}	5.44 ± 0.02^{a}	9.99 ± 1.47^{bc}	12.58 ± 1.08^{bc}	17.04 ± 0.27^{cd}	162.95 ± 1.21^{a}
RC4:0	0.22 ± 0.01^{e}	0.49 ± 0.01^{bcde}	0.61 ± 0.00^{bc}	4.95 ± 0.02^{b}	10.92 ± 1.50^{b}	11.11 ± 0.24^{cde}	17.03 ± 0.26^{cd}	112.86 ± 0.71^{b}
RC6:0	0.23 ± 0.00^{e}	0.49 ± 0.02^{bcde}	0.55 ± 0.00^{de}	4.04 ± 0.02^{g}	9.54 ± 0.40^{bcd}	10.61 ± 0.42^{de}	16.79 ± 0.35^{d}	70.23 ± 0.35^{g}
RC8:0	0.25 ± 0.03^{de}	0.48 ± 0.00^{de}	0.57 ± 0.01^{d}	3.78 ± 0.01^{h}	14.56 ± 1.07^{ab}	10.37 ± 0.33^{e}	17.46 ± 0.10^{bcd}	64.96 ± 0.65^{h}
RC10:0	0.29 ± 0.02^{cd}	0.48 ± 0.01^{bcde}	0.56 ± 0.01^{de}	3.99 ± 0.03^{g}	10.29 ± 0.15^{bc}	10.74 ± 0.20^{de}	16.97 ± 0.73^{cd}	71.58 ± 0.68^{g}
RC12:0	0.31 ± 0.01^{bc}	0.46 ± 0.01^{e}	0.54 ± 0.00^{e}	4.02 ± 0.08^{g}	9.10 ± 0.39^{bcd}	10.38 ± 0.22^{e}	18.55 ± 0.26^{ab}	66.71 ± 1.48^{h}
RC14:0	0.35 ± 0.03^{ab}	0.49 ± 0.02^{bcde}	0.57 ± 0.01^{d}	4.57 ± 0.01^{cd}	8.71 ± 0.76^{bcd}	10.88 ± 0.19^{de}	19.57 ± 0.27^{a}	87.53 ± 0.65^{d}
RC16:0	0.37 ± 0.01^{a}	0.50 ± 0.01^{abcd}	0.56 ± 0.01^{de}	4.08 ± 0.01^{g}	8.87 ± 0.69^{bcd}	13.67 ± 1.02^{ab}	18.91 ± 0.16^{ab}	70.50 ± 0.76^{g}
RC18:0	0.36 ± 0.01^{a}	0.51 ± 0.01^{abcd}	0.54 ± 0.01^{e}	4.34 ± 0.03^{f}	1.48 ± 0.81^{cd}	14.20 ± 0.60^{a}	17.81 ± 0.65^{bcd}	77.95 ± 0.49^{f}
RC18:1	0.35 ± 0.02^{ab}	0.52 ± 0.00^{abc}	0.56 ± 0.01^{de}	4.64 ± 0.01^{c}	0.77 ± 0.21^{d}	13.76 ± 0.44^{ab}	18.35 ± 0.91^{abc}	91.38 ± 0.49^{c}
REPA	0.37 ± 0.00^{a}	0.52 ± 0.01^{ab}	0.55 ± 0.01^{de}	4.53 ± 0.01^{de}	1.36 ± 0.13^{cd}	14.53 ± 0.46^{a}	18.30 ± 0.37^{abc}	84.98 ± 0.35^{e}
RDHA	0.36 ± 0.00^{a}	0.54 ± 0.01^{a}	0.59 ± 0.01^{c}	5.53 ± 0.04^{a}	1.61 ± 0.45^{cd}	14.98 ± 0.32^{a}	18.34 ± 0.95^{abc}	163.76 ± 1.09^{a}

Each value was replicated three times. Values in the same column with different letters were significantly different at $p < 0.05$ performed by Tukey's HSD test. Resveratrol (R) or its derivatives (RC3:0, RC4:0, RC6:0, RC8:0, RC10:0, RC12:0, RC14:0, RC16:0, RC18:0, RC18:1, REPA and RDHA) are resveratrol esters of propionic acid, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively.

Table 3
Inhibitory effects of resveratrol and its derivatives against *β*-carotene bleaching, LDL oxidation induced by copper, and hydroxyl radical-induced DNA scission.

Inhibition (%)	β-Carotene bleaching[1]	LDL[2]	DNA
R	71.2 ± 2.7^{a}	−32.6 ± 1.7^{e}	79.75 ± 1.86^{abc}
RC3:0	47.0 ± 1.5^{b}	44.5 ± 1.4^{c}	86.94 ± 1.40^{a}
RC4:0	43.9 ± 3.3^{b}	48.7 ± 0.7^{c}	79.76 ± 1.49^{abc}
RC6:0	23.8 ± 0.8^{cde}	42.0 ± 2.9^{c}	80.92 ± 1.31^{abc}
RC8:0	15.4 ± 0.7^{fg}	52.0 ± 7.3^{c}	86.15 ± 3.58^{ab}
RC10:0	19.0 ± 4.1^{ef}	45.7 ± 8.3^{c}	84.46 ± 3.63^{abc}
RC12:0	20.4 ± 2.1^{f}	73.2 ± 1.6^{b}	81.03 ± 1.77^{abc}
RC14:0	11.4 ± 1.8^{g}	46.2 ± 4.1^{c}	77.94 ± 2.49^{abc}
RC16:0	21.9 ± 1.0^{def}	86.4 ± 4.8^{a}	75.94 ± 0.70^{c}
RC18:0	29.4 ± 0.9^{c}	80.4 ± 4.1^{ab}	79.07 ± 3.71^{abc}
RC18:1	27.4 ± 0.4^{cd}	28.7 ± 1.5^{d}	77.55 ± 7.67^{bc}
REPA	20.2 ± 1.0^{ef}	-21.4 ± 4.4^{e}	77.23 ± 1.18^{bc}
RDHA	25.4 ± 0.5^{cde}	27.9 ± 5.9^{d}	79.38 ± 1.04^{abc}

Each value was replicated three times. Values in the same column with different letters were significantly different at P < 0.05 performed by Tukey's HSD test. Resveratrol (R) or its derivatives (RC3:0, RC4:0, RC6:0, RC8:0, RC10:0, RC12:0, RC14:0, RC16:0, RC18:0, RC18:1, REPA and RDHA) are resveratrol esters of propionic acid, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively.

[1] Inhibition (%) after 105 min incubation.

[2] Inhibition (%) after 8 h incubation.

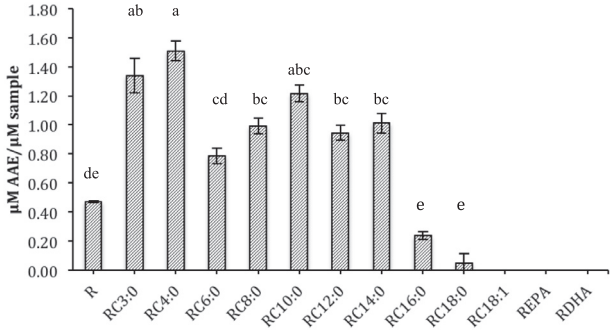


Fig. 2. Hydrogen peroxide scavenging activities in micromoles of ascorbic acid equivalents (AAE) per micromoles of resveratrol and its derivatives. Resveratrol (R) or its derivatives (RC3:0, RC4:0, RC6:0, RC8:0, RC10:0, RC12:0, RC14:0, RC16:0, RC18:0, RC18:1, REPA and RDHA) are resveratrol esters of propionic acid, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively). Bars (mean values of 3 replicates ± SD) with different letters were significantly different at $p < 0.05$ performed by Tukey's HSD test.

invading microorganisms (Chandrasekara & Shahidi, 2011). Although H_2O_2 is one of the non-radical reactive oxygen species (ROS), it can be very toxic to the cells due to its ability to cause DNA damage and membrane disruption (Halliwell, 1991). It is also possible that in the presence of iron or copper ions, H_2O_2 can form hydroxyl radical ($HO^{\bullet}$) which is a highly reactive radical (Chandrasekara & Shahidi, 2011). Therefore, it is important to keep the level of H_2O_2 in balance. In this study, H_2O_2 scavenging activity of resveratrol and its derivatives was determined, the results are shown in Fig. 2. Some resveratrol derivatives (RC3:0–RC14:0) showed a better performance than the parent resveratrol. RC3:0 and RC4:0 were 3–3.2 fold and RC6:0–RC14:0 were 1.6–2.4 fold more effective than resveratrol. Konyalioglu, Armagan, Yalcin, Atalayin, and Dagci (2013) reported that resveratrol might be effective in hydrogen peroxide-induced oxidation stress on embryonic neural stem cells. Jang and Surh (2001) stated that pheochromocytoma (PC12) cells in rats may be protected by resveratrol from hydrogen peroxide-induced apoptosis. This study suggests that resveratrol derivatives could render enhanced effects on H_2O_2 scavenging activity so that they may lead to better activities than resveratrol itself in various diseases caused by H_2O_2. Meanwhile, except RC3:0 and RC4:0, the compounds from R to RC10 exhibited an increasing trend of activity in the order of RC10:0 > RC8:0 > RC6:0 > R. However, the test compounds from RC12:0 to RC18:0 followed an opposite trend. Moreover, unsaturated esters showed no H_2O_2 scavenging activity. This might also be related to the so-called nonlinear or cutoff effect. Several studies have reported a cutoff effect in emulsion as well as other systems (Devínsky, Kopecka-Leimanová, Šeršeň, & Balgavý, 1990; Locatelli et al., 2008). Devínsky, et al. (1990) found cutoff effect on antimicrobial activity of the homologous series of N,N-dimethylalkylamine oxides for *Staphylococcus aureus* and *Escherichia coli*. Locatelli et al. (2008) observed cutoff effect on the cytotoxic effect toward L1210 leukemia cells. They reported that log P 4–5 (carbon chain 8–12) showed a higher cytotoxic activity than others.

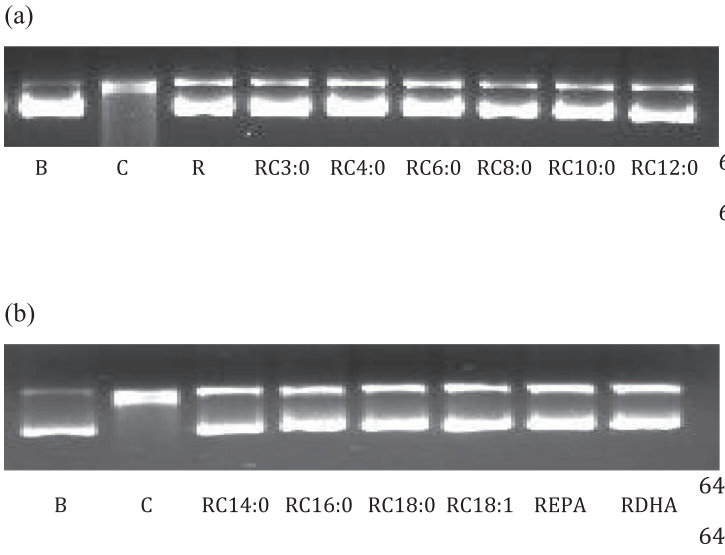


Fig. 3. Inhibition (%) of hydroxyl radical induced DNA scission by (a) R, RC3:0, RC4:0, RC6:0, RC8:0, RC10:0, and RC12:0 and (b) RC14:0, RC16:0, RC18:0, RC18:1, REPA, and RDHA (B: DNA only, C: DNA + $FeSO_4$ + H_2O_2). Resveratrol (R) or its derivatives (RC3:0, RC4:0, RC6:0, RC8:0, RC10:0, RC12:0, RC14:0, RC16:0, RC18:0, RC18:1, REPA and RDHA) are resveratrol esters of propionic acid, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, eicosapentaenoic acid, and docosahexaenoic acid, respectively.

3.5. Inhibition against copper-induced low-density lipoprotein (LDL) oxidation

LDL is an essential particle to transport cholesterol to cells in the human body. Since LDL is made of lipids such as phospholipids, cholesterols, and triacylglycerols, it is susceptible to oxidation. The oxidation of LDL might start with phospholipids located on the surface of the LDL and then propagate to the core lipids causing modification of phospholipids, lipids, cholesterols, as well as apolipoprotein B (Witztum, 1994). The oxidized LDL, known as modified LDL, can lead to atherosclerosis. Therefore, inhibition of LDL oxidation is of interest to many scientists. There are two ways to initiate LDL oxidation, one is using transition metals such as copper ion (Cu^{2+}) and the other one is using AAPH to generate peroxyl radical (Shahidi & Zhong, 2015). In this study, LDL oxidation was induced by $CuSO_4$ and the LDL oxidation (formation of conjugated dienes) was monitored spectrophotometrically at 234 nm. Resveratrol is known as an inhibitor of human LDL oxidation (Frankel, Waterhouse, & Kinsella 1993). Frankel, et al. (1993) measured hexanal formation by copper-catalyzed oxidation, in which resveratrol was able to inhibit LDL oxidation by 70–81%. However, resveratrol and resveratrol with EPA showed a prooxidative activity in this study (Table 3). There are several studies suggesting resveratrol as being responsible for DNA cleaving due to its prooxidant effect (Fukuhara & Miyata, 1998; Fukuhara et al., 2006). Fukuhara and Miyata (1998) reported that resveratrol was able to convert supercoiled DNA to nicked DNA effectively at neutral pH in the presence of O_2 and Cu^{2+}. On the other hand, resveratrol could not damage the DNA in the presence of other metal ions such as Mg^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, Co^{2+} or Fe^{3+}. Although Frankel et al. (1993) did not report specific condition for their experiment reporting inhibitory effect of LDL oxidation, the pro-oxidative activity of resveratrol in this work might originate from different conditions such as pH and presence of O_2. The behaviour of resveratrol (antioxidant or prooxidant) in LDL oxidation induced by copper needs to be further investigated. Interestingly, most resveratrol derivatives were able to inhibit LDL oxidation. Among resveratrol derivatives, RC16:0 and RC18:0 showed the highest inhibition of LDL oxidation. Structural modification might affect the inhibition ability of test materials. Fukuhara et al. (2006) studied the structural basis of resveratrol and its analogues for DNA-cleaving activity in the presence of Cu^{2+}. Among hydroxyl groups of resveratrol, 4′-hydroxyl group is most important for DNA cleavage due to its ability to bind with Cu^{2+}. Therefore, loss of hydroxyl groups via esterification could affect to their binding ability with Cu^{2+}, which leads to a better inhibition of LDL oxidation.

3.6. Inhibition against hydroxyl radical-induced DNA scission

It is well known that damaged DNA, occurred by reactive oxygen species (ROS), can lead to mutation and carcinogenesis. However, ROS is not avoidable to living cells because it is continuously generated via biochemical reactions and external factors (Loft & Poulsen, 1996). For example, hydroxyl radical, one of the ROS, can be generated from degradation of hydrogen peroxide through Fenton reaction and from interaction between superoxide and hydrogen peroxide via Haber-Weiss reaction (Yu, 1994). Although it is the most reactive radical of the ROS, it has low diffusion capability due to its short half-life. Antioxidative defense systems to protect from ROS in our body such as superoxide dismutase, GSH peroxidase, and catalase are frequently inadequate (Yu, 1994; Loft & Poulsen, 1996). Therefore, it is of interest to find external sources of antioxidant. Antioxidants, critical barriers against free radical damage, are usually used to prevent oxidative damage. In this study, hydroxyl radical was employed to determine the inhibition of DNA scission by resveratrol and its derivatives. Hydrogen peroxide and Fe^{2+} were used to generate hydroxyl radical. As shown in Fig. 3, supercoiled DNA in the control was fully converted into open circular DNA by hydroxyl radicals, however the linear form was not observed. Resveratrol esters were able to inhibit DNA scission with insignificant difference compared to resveratrol itself ($p > 0.05$, Table 3). The esterification did not affect the inhibition of DNA scission in this work. However, Zhou, Sun, and Shahidi (2017) reported that esterification of tyrosol increased inhibition of DNA scission induced by hydroxyl radical, whereas esterification of hydroxytyrosol decreased their inhibition of DNA scission. According to Zhong and Shahidi (2012), inhibition against DNA scission of EGCG and its derivatives might be due to a combination of radical scavenging and Fe^{2+} chelation. Some studies have reported that resveratrol also has Fe^{2+} chelating ability (Hussein, 2011; Gülçin, 2010). Hence, inhibition against DNA scission induced by hydroxyl radical might also be due to a cumulative effect of radical scavenging, Fe^{2+} chelating ability, and H_2O_2 scavenging ability. Although resveratrol derivatives (RC3:0–RC14:0) showed a significantly better H_2O_2 scavenging activity than resveratrol, inhibition against DNA scission induced by hydroxyl radical was to various extent but not significantly different. This might be due to its compromised radical scavenging ability. Resveratrol derivatives showed a lower radical scavenging activity in both DPPH radical and ABTS radical cation scavenging assays (Oh & Shahidi, 2017), suggesting that losing one or more hydroxyl group might compromise their radical scavenging activity. Therefore, decreased radical scavenging activity might counteract the better effect of resveratrol derivatives (RC3:0–RC14:0) on

H_2O_2 scavenging ability. While resveratrol unsaturated derivatives (RC18:1, REPA, and RDHA) showed no H_2O_2 scavenging activity; they showed similar inhibition of DNA scission induced by hydroxyl radical compared to resveratrol. This might be due to structural nature (bent structure) of unsaturated fatty acids that might positively influence to the metal chelation activity (Zhong & Shahidi, 2012).

4. Conclusion

This study demonstrated the potential extended use of lipophilized resveratrol derivatives in food and biological systems to control oxidative processes. The derivatives showed a range of antioxidant activities depending on the test model system employed. We observed disagreement with polar paradox in both oil-in-water emulsion and bulk oil systems. Moreover, nonlinear phenomenon was observed in bulk oil system and hydrogen peroxide scavenging activity. The test compounds, except resveratrol and REPA, were able to effectively inhibit LDL oxidation induced by copper ion. In addition, all test compounds inhibited DNA scission induced by hydroxyl radical. The results clearly demonstrated that resveratrol derivatives might serve as potential antioxidants in food and biological systems.

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