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RESEARCH ARTICLE

Revisiting polar paradox: antioxidant activity in bulk oil using selected phenol lipids

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Abstract

The purpose of this work was to re-evaluate the polar paradox theory (PPT) that explains the relationships between the efficacy of antioxidants, their polarity, and their environments. In this study, ascorbic acid (AA), ascorbyl palmitate (AP), gallic acid (GA), gallyl palmitate (GP), Trolox (TR), α-tocopherol (TO), resveratrol (R), and resveratryl palmitate (RP) were employed to assess conjugated dienoic acid (CDA), the *p*-anisidine value (*p*-AV), headspace oxygen content, and hexanal formation in a bulk oil system. TR, TO, R, and RP showed better antioxidant activities in CDA and *p*-AV and higher headspace oxygen content than AA, AP, GA, and GP. AA showed lower hexanal formation than AP, whereas GP, TO, and RP had better antioxidant activity than their derivatives. These findings suggest that the PPT might be useful to explain the oxidation that occurs at the air-oil interface/association colloids but applying it to other assays might not appropriate.

Keywords Phenol lipids · Lipophilization · Antioxidant activity · Polar paradox theory

Introduction

Lipid oxidation is a major deteriorative process, which is responsible for off-flavor in lipid-containing foods. One of the main strategies to prevent its oxidation is the addition of antioxidants. This is the most effective, convenient, and economical method. Thus, it is frequently employed in the food industry (Senanayake et al., 2020). Antioxidants can delay the onset or rate of oxidation by scavenging free radicals and reactive oxygen species, quenching singlet oxygen, and chelating metal ions, among others (Lü et al., 2010). Many factors can affect the effectiveness of antioxidants, such as the concentration of antioxidants, the type of oxidation substrate and antioxidants, and the physical state of the system media (Shahidi and Zhong, 2011). Thus, explaining or anticipating the behavior of antioxidants is challenging.

Antioxidants often showed paradoxical behavior in various media. Polar antioxidants showed better activity in a lipophilic system than non-polar ones. In contrast, non-polar antioxidants are more effective than polar chemicals in oil-in-water emulsion systems (Oh et al., 2023). This phenomenon is called the "polar paradox theory." There are several attempts to apply the theory to bulk oil or food systems. Li et al. (2015) used bulk oil as a solvent to extract hydrophilic antioxidants from olive leaves using different types of surfactants (soja lecithin, diglycerides, unsaturated or saturated monoglycerides). Huber et al. (2009) reported that quercetin and its glycosides showed better inhibition of lipid oxidation than butylated hydroxytoluene (BHT; hydrophobic antioxidant) in bulk fish oil. Another study group (Lee and Surh, 2021) used carrot powder, which contains a high amount of hydrophilic antioxidants, to delay lipid oxidation during deep-frying croquettes, whereas non-polar antioxidants in curry powder were not effective in bulk oil during croquette preparation in agreement with the polar paradox theory (Jo and Surh, 2020).

As mentioned above, the polar paradox theory has been extensively studied, confirmed, and generally used to explain the results of antioxidant studies. However, several studies

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observed results contradicting this theory (Shahidi and Zhong, 2011). Four phenolic compounds, namely (−)-epigallocatechin (EGC) (Ambigaipalan et al., 2020a), epigallocatechin gallate (EGCG) (Zhong and Shahidi, 2012), resveratrol (Oh and Shahidi, 2018), and quercetin (Oh et al., 2021) have been lipophilized and the antioxidant activity was compared between the parent molecule and their derivatives. EGC and EGCG, which are water-soluble phenolic compounds, showed lower antioxidant activity than their derivatives in oil-in-water emulsions. These observations followed the polar paradox theory. In contrast, resveratrol and quercetin, which possess low solubility in water, had lower antioxidant activity in bulk oil systems. This result disagreed with the theory. Thus, it was hypothesized that water-soluble antioxidants would follow the polar paradox theory, whereas water-low/insoluble ones would show contradictory results. This study employed two water-soluble antioxidants (ascorbic acid (AA) and gallic acid (GA)) and their derivatives (ascorbyl palmitate (AP) and hexadecyl gallate (gallyl palmitate, (GP)), as well as two water low/insoluble antioxidants (Trolox (TR) and resveratrol (R)) and their derivatives (α-tocopherol (TO) and resveratryl palmitate (RP)) to test the hypothesis.

Materials and methods

Materials

Corn oil was purchased from a local grocery market (Suwon, Korea). GA, GP, R, and vinyl palmitate were obtained from the Tokyo Chemical Industry (TCI). Novozyme 435 and 2-methyl-2-butanol were procured from Novozymes (Bagsvaerd, Denmark) and Acros Organics (Germany), respectively. Thin-layer chromatography (TLC) with silica gel 60 F_{254} (2.5 × 7.5 cm) and silica gel 60 were bought from Merck (Darmstadt, Germany). AA, AP, TR, TO, silicic acid, and activated charcoal were purchased from Sigma-Aldrich (St. Louis, MO, USA), and other chemicals were obtained from Daejung Chemical Co. (Seoul, Korea).

Preparation of resveratryl palmitate

Detailed procedures for the preparation of RP were published previously (Torres et al., 2010). Briefly, resveratrol (57 mM), vinyl palmitate, and 5 mL of 2-methyl-2-butanol in nitrogen-filled and sealed amber bottles were kept at 40 °C in the dark in a shaking water bath (BS-31, JEIO Tech., Seoul, Korea) at 150 rpm for 48 h. The reaction was confirmed by HPLC-photodiode array (PDA, PM1430, Hitachi Co., Tokyo, Japan) as well as HPLC (Ultimate3000; Thermo Scientific, USA) coupled with electrospray (ESI)-triple time of flight (TOF; AB SCIEX, USA).

The RP produced was separated from intact resveratrol by column chromatography according to the method described by Oh and Shahidi (2017). The stationary phase was silica gel, and the mobile phase was a hexane/ethyl acetate/formic acid gradient elution (90:10:2–60:40:2, v/v/v). Separation was monitored by TLC plates and an HPLC-photodiode array (PDA, PM1430, Hitachi Co., Tokyo, Japan).

Oxidation inhibition measurements using stripped corn oil

Preparation of stripped corn oil

A simplified stripping process was carried out in corn oil to remove minor compounds. The activation of silicic acid was carried out according to the method of Abad and Shahidi (2020) with a slight modification. Approximately 100 g of silicic acid and 1 L of distilled water were stirred for 1 h then left to stand for 30 min to settle. The supernatant was discarded, and this was repeated three times. Finally, the silicic acid was washed using methanol, suction-filtered, and kept in a drying oven at 110 °C overnight.

The corn oil with hexane (1:10, w/v) was stirred with activated silicic acid (180 g) and charcoal (90 g) for an hour under a nitrogen steam in the dark. The eluting solvent was collected via filtration and then evaporated.

Analysis of conjugated dienoic acid and p-anisidine value

Stripped corn oil (1 g) prepared with a stock solution (100 μM, 100 μL) dissolved in ethanol was transferred to a brown vial under nitrogen purging. The samples were then kept in an oven at 60 °C and measurements were made on days 0, 2, 4, 6, and 8. The CDA and *p*-AV were conducted according to the American Oil Chemists' Society (AOCS) method Ti la-64 and Cd 18–90, respectively (AOCS, 2006a, b).

Rancimat analysis

Rancimat analysis was performed as described by Park et al. (2021). Stripped corn oil (3 g) and samples in ethanol (100 μL, 50 μM) were transferred to Rancimat tubes under a nitrogen blanket. After that, the tubes were placed in the Rancimat instrument (Metrohm, Chennai, India) at 100 °C.

Headspace oxygen content analysis

Headspace oxygen content was measured as described by Yoo et al. (2023). Corn oil (1 g) and sample (100 μM, 100 μL) dissolved in ethanol were transferred to brown vials and kept in an oven at 60 °C. The samples were taken on days 0, 2, 4, 6, and 8 and then subjected to a gas

chromatography-thermal conductivity detector (GC-TCD; Agilent Technologies Inc 7890, Santa Clara, CA, USA). Headspace gas (30 µL) was taken into the GC-TCD. The inlet temperature was 60 °C, and helium carrier gas flowed at a rate of 200 mL/min. The stationary phase was a 60/80 packed column (3.0 m × 2 mm internal diameter; Restek Ltd., PA, USA). The oven and detector temperature was set to 180 °C.

Hexanal measurement using solid phase micro-extraction gas chromatography-mass spectrometry

Corn oil (1 g) and sample (100 µM, 100 μL) dissolved in ethanol in brown vials were placed in an oven at 60 °C for 8 days. The headspace volatile compounds were extracted using solid phase micro-extraction gas chromatography-mass spectrometry (SPME) and then subjected to GC–MS. The SPME and GC–MS conditions followed a method described by Kim et al. (2023). The samples were placed in an agitator in a multipurpose sampler after standing in a water bath for 30 min at 30 °C. The SPME fiber used was a Polydimethylsiloxane /Divinylbenzene (PDMS/DVB) fiber (65 µm; Supelco; Bellefonte, PA, USA). The fiber extracted the volatile compounds for 30 min at 30 °C. A 6890 GC unit connected to a 5971A mass selective detector (MS) (Agilent Technologies) was employed for GC–MS analysis. The mobile phase and stationary phase were helium (1.0 mL/min) and a DB-5 ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Agilent J & W; Folsom, CA, USA), respectively. The oven temperature was programmed from 40 °C (3 min) to 150 °C (4 °C/min) and then to 220 °C, with a heating rate of 15 °C/min. The ion source was at 70 eV, and the interface was held at 220 °C. Alkane standards (C6-C20) were employed to determine the retention index (RI). National Institute of Standards and Technology (NIST) mass and alkane standards were used for data analysis.

Statistical analysis

Duncan's multiple range test with analysis of variance (ANOVA) at a p-value of < 0.05 was employed. SPSS software program version 19 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analyses.

Results and discussion

Preparation of RP

Preparation of R ester can be achieved either chemically or enzymatically. Previous studies on EGC (Ambigaipalan et al., 2020b), quercetin (Oh et al., 2019), and R (Oh and Shahidi, 2017) used chemical methods. Although the chemical procedure is reliable and straightforward, it has serious implications, such as by-products and difficulty in controlling the esterification position. Unlike the chemical method, enzymatic esterification can control esterification due to the regioselectivity of the enzymes, and it produces fewer by-products. Since R possesses three hydroxyl groups, it has different antioxidant activity depending on the position of the esterification and the number of esterifications that occur. Wang et al. (2018) reported that lipases PSL (3-*O*-monoester > 4′-*O*-monoester > diester), CSL (4′-*O*-monoester > diester), AKL (diester > 3-*O*-monoester ≈ 4'-*O*-monoester), and CAL-B (4′-*O*-monoester > diester > 3-*O*-monoester) produced different types of resveratrol esters. Another study found that Novozyme 435 synthesized 4'-*O*-monoester (Kuo et al., 2013). Torress et al. (2010) reported that 3-OH seemed more important in terms of antioxidant activity (Fig. 1a). They also reported that the R triester showed the lowest antioxidant activity in TR equivalent antioxidant capability, followed by diester and monoester. Thus, Novozym 435 was employed for this study.

After enzymatic esterification, the intact molecule was separated from the products by column chromatography and was monitored by TLC and HPLC–PDA. According to the HPLC–PDA results, the esterification yield was 28.55 ± 1.20% (Fig. 1b). The esterification yield could have been influenced by the chain length of the fatty acid vinyl ester. The esterification between R and vinyl acetate using Novozym 435 was around 60–80% (Kuo et al., 2013; Torress et al., 2010). In contrast, the yield when using vinyl stearate was a maximum of 35% (Torress et al., 2010). A similar phenomenon was observed in chemical esterification. According to Oh and Shahidi (2017), a yield of 74% was achieved by the esterification of R using propionyl chloride, whereas the incorporation yield of R with docosahexaenoyl chloride was 37.7%. They reported that this could be due to the long-carbon chain and nonlinear characteristics of DHA. The esterification was confirmed by HPLC–MS. RP was detected at m/z 467.3147, which indicated that the product was a monoester (Fig. 1c).

Primary and secondary oxidative product measurements

A stripping process is used to remove minor compounds that exist in bulk oil. The minor compounds include diacylglycerols (DAG), monoacylglycerols (MAG), free fatty acids (FFA), phospholipids (PLs), sterols, chlorophylls, carotenoids, tocols, water, and minerals (Chen et al., 2011). Stripped corn oil was used to examine the antioxidant activity in bulk oil using selected phenol lipids. CDA formation and p-AVs, which are considered primary and secondary

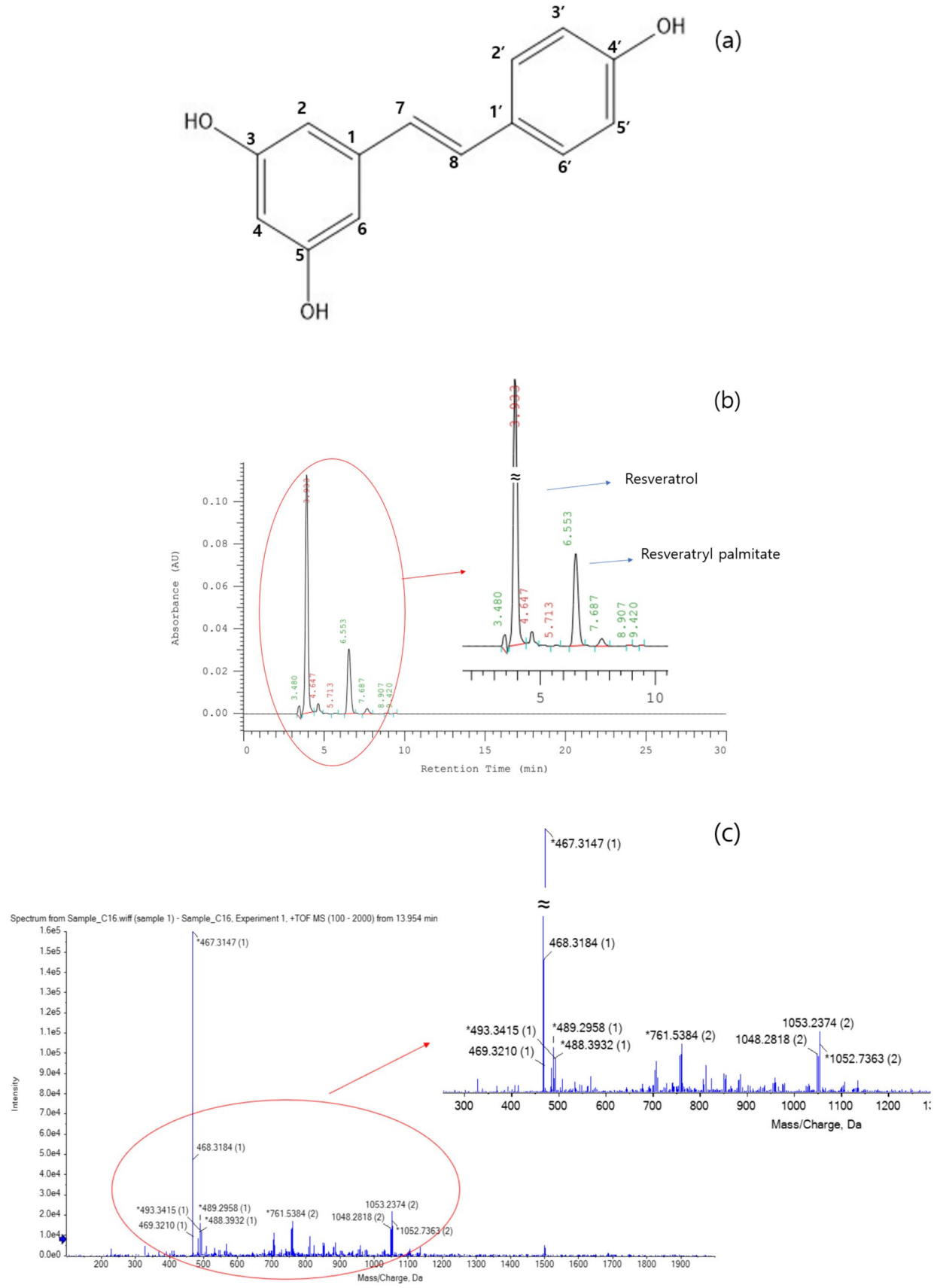

Fig. 1 Structure of resveratrol (**A**), chromatogram of resveratrol and resveratryl palmitate (**B**), and mass spectra of resveratryl palmitate (**C**)

oxidative products, respectively, were measured on days 0, 2, 4, 6, and 8 of storage. CDA and *p*-AVs on days 4 and 6, respectively, are shown in Fig. 2. All antioxidants had significantly lower CDA levels compared to the control except for RP, whereas all antioxidants showed lower *p*-AVs than the control. Although RP had the least antioxidant activity in CDA, it showed antioxidant activity similar to the A group (AA and AP) measured by *p*-AV. Two water-low/insoluble antioxidants and their derivatives showed better antioxidant activity than the water-soluble antioxidants and their derivatives in both assays. However, no significant pattern was observed in a comparison between the parent and lipophilized molecules. This might be due to the limited number of esterification. All derivatives used in this study were 16 carbon chains and monoesters to control the effect of chain length and number of esterifications. In another study, resveratrol esters showed better antioxidant activity than resveratrol in bulk oil (Oh and Shahidi, 2018). That study used resveratrol esters as a mixture of resveratrol monoester and diester. Also, the other antioxidants mentioned above were mono, di, and triester of EGC (Ambigaipalan et al., 2020b), mono, di, tri, and tetraester of quercetin (Oh et al., 2019), and tetraester of EGCG (Zhong and Shahidi, 2012). Thus, a number of esterification needs to be investigated further to revisit the polar paradox theory.

Analysis of the induction period

Rancimat is an instrument for mimicking deep-fat frying and lipid oxidation. The oil placed in the Rancimat experiences heat and aeration to produce oxidative products, such as polar compounds, organic acids, odor components, and viscosity (Perkins, 1992; Zhong and Shahidi, 2012). These are detected, and the result is used as an indicator of oxidation. The induction period measured by the Rancimat is shown in Fig. 3. The G group (GA and GP) showed significantly higher antioxidant activity than other antioxidants. AP and RP showed slightly higher induction times than

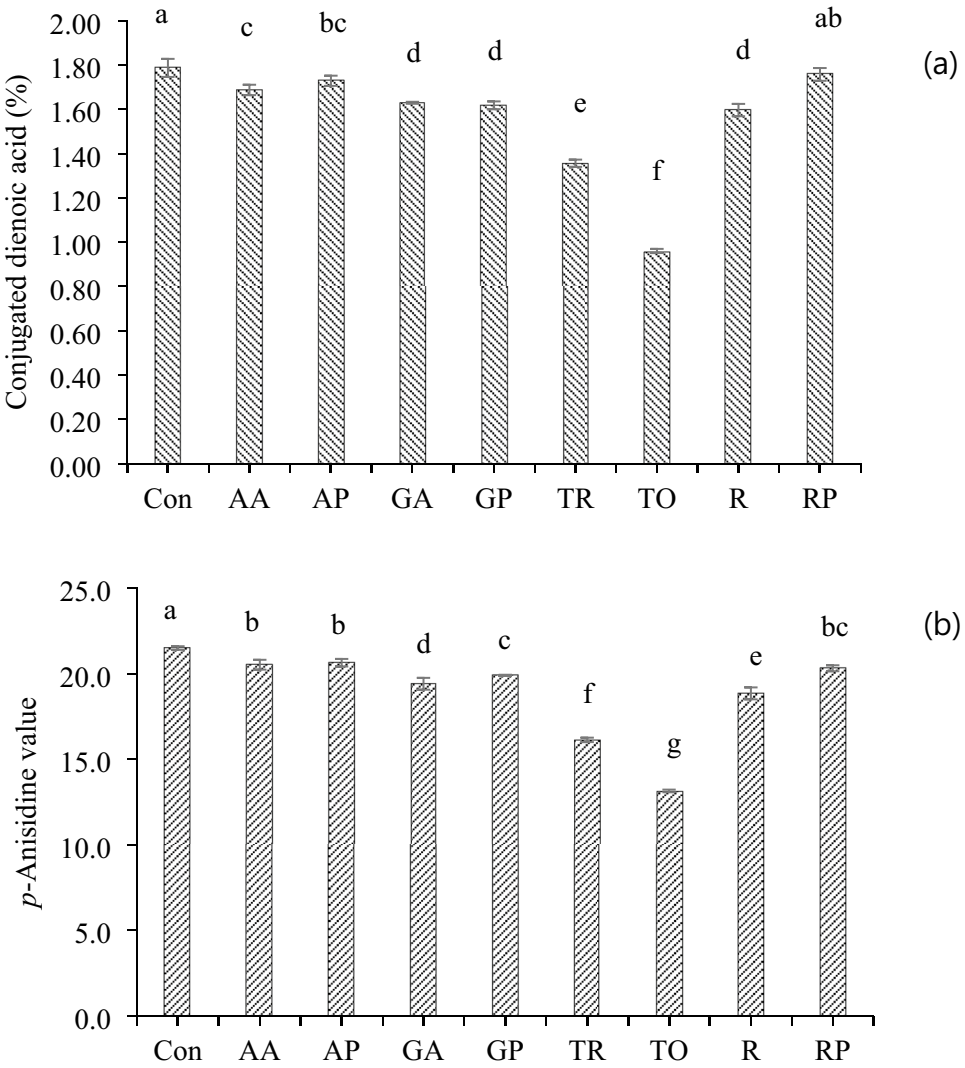

Fig. 2 Conjugated dienoic acid (**A**) and *p*-anisidine value (**B**) of stripped corn oil on days 4 and 6 of the storage period, respectively. Con, AA, AP, GA, GP, TR, TO, R, and RP are corn oils without the addition of antioxidants, oils with ascorbic acid, ascorbyl palmitate, gallic acid, hexadecyl gallate, Trolox, α-tocopherol, resveratrol, and resveratryl palmitate, respectively. The different letters are significantly different ($P < 0.05$) performed by Duncan's multiple range test. Each value was replicated three times

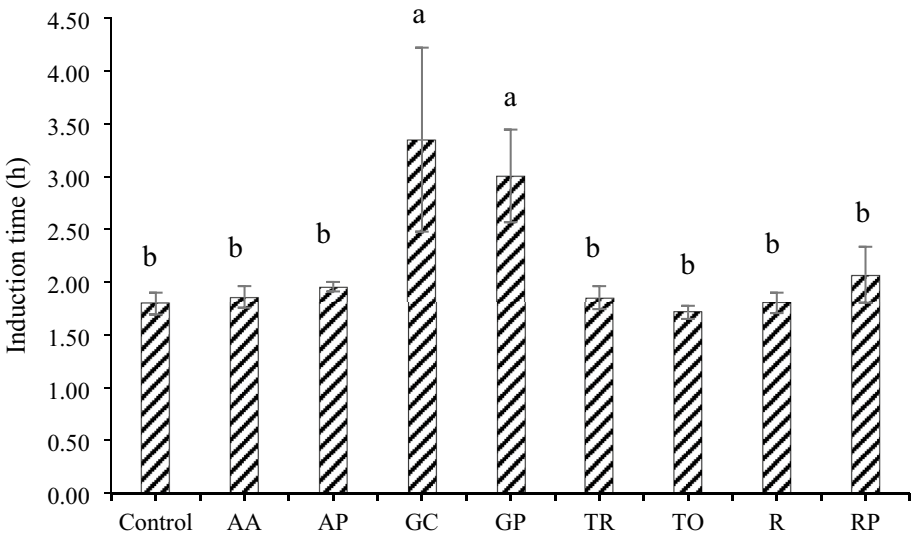

Fig. 3 Rancimat assay of stripped corn oil containing antioxidants. Abbreviations are listed in the legend of Fig. 2. The different letters are significantly different ($P < 0.05$) performed by Duncan's multiple range test. Each value was replicated three times

other samples and control. However, no significant differences were seen. It was hypothesized that the lipophilized derivatives of two water-low/insoluble antioxidants (TO and RP) would show better antioxidant activity than their parent molecules. In terms of water-soluble antioxidants, parent molecules would show higher oxidative stability than their derivatives. However, only the G group (GC and GP; water-soluble antioxidants) and the R group (R and RP; water-low/insoluble antioxidants) exhibited the expected trend. This might have been due to different oxidation temperatures. Although the chemical mechanism of thermal oxidation shares the same oxidation process of autoxidation, thermal oxidation has a more complex and faster oxidation mechanism (Choe and Min, 2007). Besides the typical radical chain reaction, thermal oxidation undergoes hydrolysis due to the presence of water. The Rancimat monitors changes in conductivity influenced by polar compounds and organic acids (García-Moreno et al., 2013; Woo et al., 2019).

Headspace oxygen content

Lipid oxidation is a chemical reaction between lipids and oxygen. A decreased in headspace oxygen content may indicate lipid oxidation. The headspace oxygen content results are shown in Fig. 4. All antioxidants used showed higher headspace oxygen levels than the control. Among antioxidants, the R group (R and RP) exhibited the highest headspace oxygen content, whereas the A group (AA and AP) showed the lowest. Except for the G group (GA and GP), AP, TO, and RP showed lower antioxidant

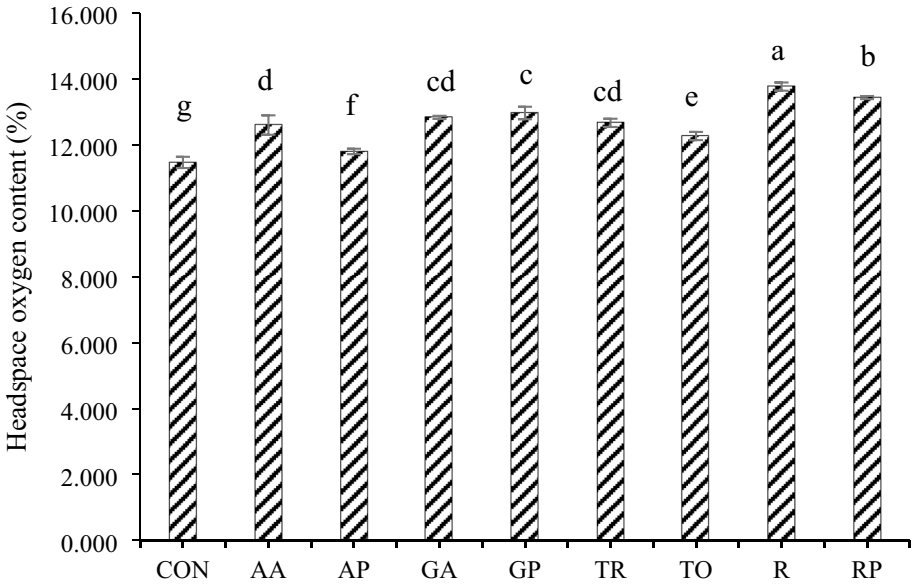

Fig. 4 Changes of headspace oxygen in corn oil containing antioxidants over an 8-day storage period. Abbreviations are listed in the legend of Fig. 2

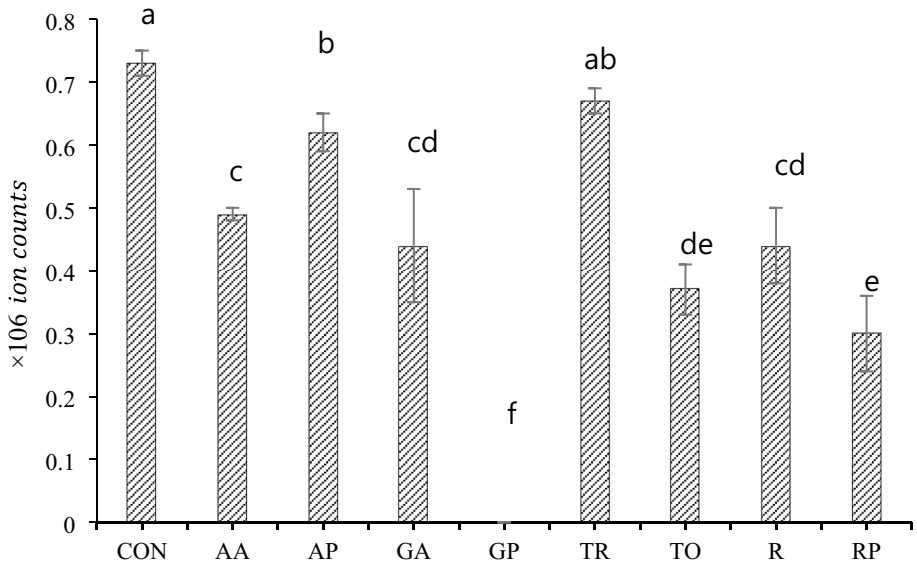

Fig. 5 1-Hexanal level in corn oil containing antioxidants over an 8-day storage period. Abbreviations are listed in the legend of Fig. 2

activity than AA, TR, and R. Oxygen placed in the interface between the oil and air can effectively participate in the lipid oxidation process. Thus, antioxidants need to be on the surface of the oil to efficiently prevent oxidation. Antioxidants without side chains (AA, TR, and R) might be placed on the surface of the oil, whereas antioxidants with side chains (AP, TO, and RP) are in the oil. These results agree with the polar paradox theory, which states that polar antioxidants tend to be more effective than non-polar antioxidants in bulk oil.

Interfacial phenomena are a possible explanation for the polar paradox theory. According to this explanation, the polar antioxidants in bulk oil concentrate at the air-oil interface or associated colloids where oxidation actively occurs. In contrast, non-polar antioxidants placed in the oil are far from where oxidation occurs. Thus, these are less effective (Laguerre et al., 2015). In terms of oil-in-water emulsion, the antioxidants would show reverse action. An oil-in-emulsion system also needs to be tested to confirm the results. For the side chain length of antioxidants, a possible mechanism is critical chain length (CCL). This was proposed to explain the so-called nonlinear theory or cut-off effect. When the chain length is too short, the antioxidants in an oil-in-water emulsion stay throughout the water phase. With the right chain length, antioxidants can be on the surface of associated colloids. This chain length is called CCL. At this point, the antioxidants show the maximum efficacy to inhibit oxidation. The antioxidants with side chains beyond the CCL can be either inside the associated colloids or undergo self-aggregation and form a micelle (Laguerre et al., 2015). In general, CCL has an 8–12 carbon chain length. However, the antioxidants used in this work had 16 carbon chains. Thus, the chain length effect needs to be investigated to confirm the results of this work.

Analysis of 1-hexanal using SPME GC–MS

Linoleic acid (C18:2, around 56%) is the most unsaturated fatty acid in corn oil (Lee and Ahn, 2003). The oxidation of linoleic acid is initiated by the removal of hydrogen atoms attached to bis-allylic carbon and the subsequent formation of hydroperoxides. The hydroperoxides tend to decompose and generate secondary oxidative products, such as aldehydes, ketones, and hydrocarbons (Choe and Min, 2006). Among secondary oxidative products, hexanal is a predominant oxidative product generated from linoleic acid. Therefore, it is frequently monitored to determine the lipid oxidation rate (Kim et al., 2022). In this study, hexanal levels were measured using SPME–GC–MS. The 1-hexanal levels in corn oil containing antioxidants over an 8-day storage period are shown in Fig. 5. All test compounds except for TR showed significantly lower 1-hexanal levels compared to the control. Among the test compounds, TR exhibited the lowest antioxidant activity, whereas GP had the highest inhibition of 1-hexanal formation. While AA showed higher antioxidant activity than its derivative (AP), antioxidants with side chains (GP, TO, and RP) had better antioxidant activity than their derivatives. Shahidi research group observed similar results when using EGCG (Shahidi and Zhong, 2011), EGC (Ambigaipalan et al., 2020a), resveratrol (Oh and Shahidi, 2018), and quercetin (Oh et al., 2021). A previous study (Oh et al., 2021) reported that the lipophilicity of antioxidants plays an important role in the antioxidant activity pattern in the polar paradox theory. The study reported that phenolic compounds with lipophilicity greater than 1 would show lower antioxidant activity than their lipophilized derivatives in lipophilic media. Indeed,

the lipophilicities of GA, TO, and R were 1.17, 2.75, and 2.57, respectively.

In conclusion, the product of enzymatic esterification using resveratrol and palmitic acid was 4'-O-monoester. Among antioxidant activity tests, only the headspace content results agreed with the polar paradox theory. All other studies exhibited unrelated or contradictory results. This work demonstrates that the polar paradox theory may work for antioxidant activity measurements, which are related to the air-oil interface or associated colloids. However, further investigation is needed.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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