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Perspective

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# Antioxidant Behavior in Bulk Oil: Limitations of Polar Paradox Theory

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**ABSTRACT:** The polar paradox theory that explains the efficacy of antioxidants as affected by their polarity and that of the medium involved was re-evaluated. For the first time, the effect of concentration on validity of the polar paradox theory was investigated using four pairs of polar and nonpolar representative antioxidants in bulk oil. A model on antioxidant behavior in response to their polarity is proposed.

**KEYWORDS:** *polar paradox, antioxidant, bulk oil*

## INTRODUCTION

The polar paradox, a theory that rationalizes the paradoxical behavior of antioxidants in different media, states that polar antioxidants are more effective in less polar media, such as bulk oils, whereas nonpolar antioxidants are more effective in relatively more polar media, such as oil-in-water emulsions or liposomes.[1] The polar paradox was explained by the interfacial phenomenon,[2,3] which turned the paradox from an empirical observation into a putative theory. The theory, however, has recently been re-evaluated, after being used for two decades for the interpretation of antioxidant efficiency, as contradictory evidence from more comprehensive assessments has emerged. We have previously reported on a critical re-evaluation of the polar paradox, in which complex factors, in addition to polarity, affecting the antioxidant activity were discussed.[4] Whereas most of the studies reporting on the limitations of the polar paradox have focused on the effect of length of the hydrophobic chain on antioxidant activity of phenolipids (cutoff effect),[5] we have suggested, for the first time, that concentration plays a critical role; that is, the polar paradox may be applicable only over certain concentration ranges. For example, when the antioxidant activity of epigallocatechin gallate (EGCG) was compared to its lipophilic ester derivative (tetrastearate) in bulk oil, it was found that, at lower concentrations, the ester was more effective, whereas EGCG was more active at higher concentrations. It is hypothesized that at low concentrations the effect of solubility in bulk oil dominates over the effect of interfacial phenomenon on antioxidant efficiency; thus, nonpolar antioxidants with better fat solubility have greater efficacies than their polar counterparts, whereas the reverse is true at higher concentrations. It is therefore possible that the polar paradox theory applies only when the concentration of the antioxidant is over a critical value, so that interfacial phenomenon dominates over the solubility parameter.

We here propose an antioxidant behavior model for polar and nonpolar antioxidants in bulk oil, as illustrated in Figure 1. The bell-shaped curves indicate that the antioxidant activity increases with increasing concentration until a maximum activity is reached at the optimal concentration, after which the activity decreases with further concentration increase. As shown in Figure 1, below the critical concentration, the broken line is above the solid line, indicating higher antioxidant activity for nonpolar antioxidants than their polar counterparts. While above the critical concentration, the solid line is above the broken line, indicating an opposite trend. The polar paradox appears to reflect only the situation when antioxidant concentration is above the critical concentration while overlooking its behavior at lower concentrations, that is, below the critical concentration.

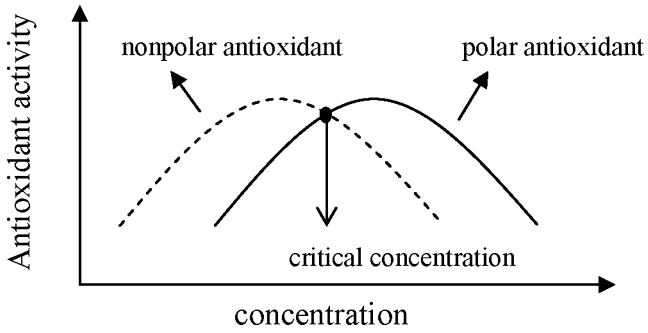


**Figure 1.** Hypothesized model for antioxidant behavior in bulk oil.

To further confirm the hypothesized model, we assessed the antioxidant activity of four sets of antioxidants in bulk oil, each set containing a hydrophilic (polar) antioxidant and its hydrophobic (nonpolar) counterpart. Preliminary results are presented, which may shed light on a better understanding of antioxidant behavior in various lipid media.

## MATERIALS AND METHODS

**Materials.** Four sets of antioxidants (each containing one hydrophilic antioxidant and its hydrophobic counterpart) were tested for their activity in bulk oil (stripped corn oil). The antioxidants tested included Trolox/$\alpha$-tocopherol, ascorbic acid/ascorbyl palmitate, gallic acid/lauryl gallate, and EGCG/EGCG-tetrastearate. Stripped corn oil, $\alpha$-tocopherol, ascorbic acid, ascorbyl palmitate, gallic acid, and lauryl gallate were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Trolox was purchased from Acros Organics (Fair Lawn, NJ). EGCG was supplied by GlaxoSmithKline Consumer Healthcare (Parsippany, NJ). Stearoyl chloride was purchased from Nu-chek Prep Inc. (Elysian, MN). EGCG-tetrastearate was synthesized as described

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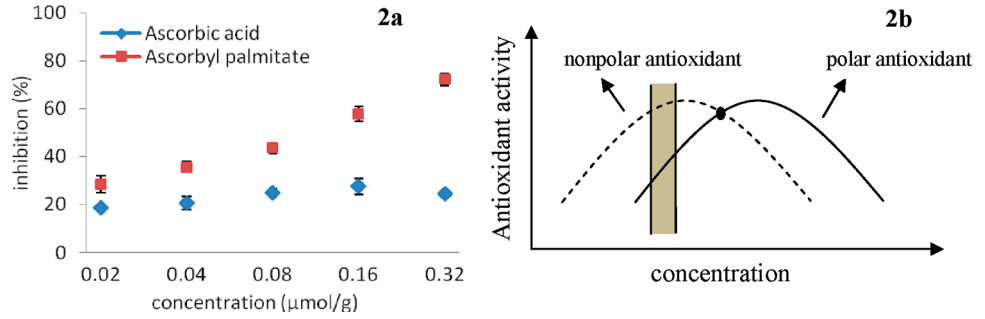


**Figure 2.** Antioxidant activity of ascorbic acid/ascorbyl palmitate in stripped corn oil.

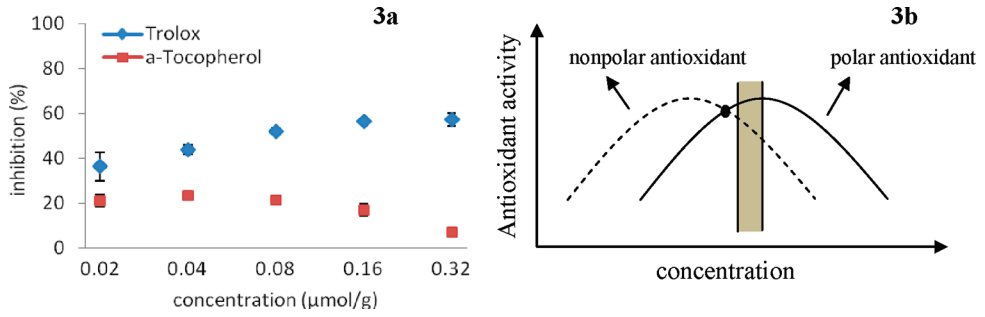


**Figure 3.** Antioxidant activity of Trolox/$\alpha$-tocopherol in stripped corn oil.

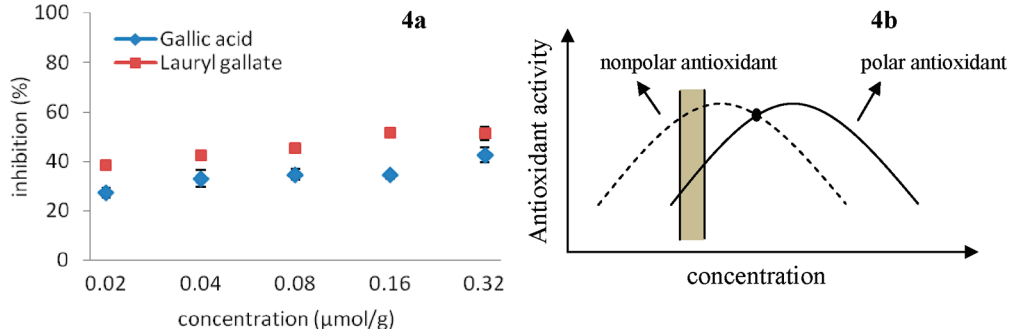


**Figure 4.** Antioxidant activity of gallic acid/lauryl gallate in stripped corn oil.

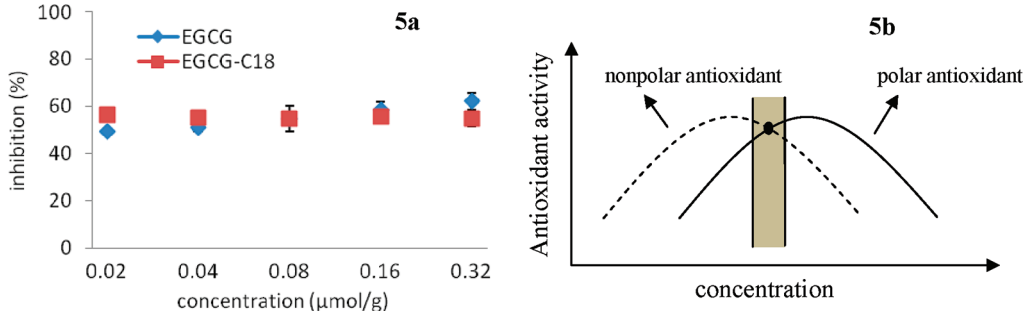


**Figure 5.** Antioxidant activity of EGCG/EGCG-tetrastearate in stripped corn oil.

below. All solvents used were obtained from Fisher Scientific Ltd. (Ottawa, ON, Canada). The solvents employed were of HPLC or reagent grade.

**Preparation of EGCG-tetrastearate.** EGCG-tetrastearate was prepared by esterification of EGCG with stearoyl chloride, according to a previously reported procedure.[6] The crude product containing a mixture of EGCG polyesters was separated by flash column chromatography, and the major ester compound (tetraester) was collected and identified by HPLC-MS, $^{1}$H NMR, and $^{13}$C NMR.[6]

**Antioxidant Activity Assessment in Bulk Oil.** The four sets of antioxidants were evaluated for their activity in a stripped corn oil over a concentration range. The antioxidants were added to stripped corn oil at a series of concentrations (0.02, 0.04, 0.08, 0.16, and 0.32 $\mu$mol/g oil), and the oil was stored under Schaal oven conditions at 60 °C for 7 days. The oxidative status of the oil was indicated by its content of conjugated dienes (CD).

The CD values were determined by measuring the absorbance of the oil dissolved in iso-octane at 234 nm and calculated according to the equation CD = absorbance/$cd$, where $c$ and $d$ stand for the


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concentration of the oil and the length of the cuvette, respectively. The antioxidant activity was calculated as percentage inhibition in CD formation as compared with the control.

## RESULTS AND DISCUSSION

The results of the activity of each set of antioxidants are shown in Figures 2−5. For the ascorbic acid/ascorbyl palmitate set (Figure 2), ascorbyl palmitate had higher antioxidant activity than its polar counterpart ascorbic acid over the full concentration range of 0.02−0.32 μmol/g, which represents the highlighted region in Figure 2b of the proposed model. This suggests that over the test concentration ranges, the antioxidant activity of ascorbic acid and ascorbyl palmitate is not in agreement with the polar paradox, in which the opposite trend is predicted. The results also suggest that the critical concentration of these two antioxidants may be above 0.32 μmol/g, as can be deduced from Figure 2b.

Figure 3 shows the antioxidant efficacy of Trolox/α-tocopherol in the stripped corn oil. The antioxidant activity of Trolox increased with increasing concentration, whereas the opposite trend was observed for α-tocopherol. Different from ascorbic acid/ascorbyl palmitate, the polar Trolox was more effective than the nonpolar α-tocopherol, which is in agreement with the polar paradox. Therefore, the tested concentration range may be reflected as the highlighted region in Figure 3b, and it is possible that the critical concentration for Trolox/α-tocopherol may be below 0.02 μmol/g.

For the gallic acid/lauryl gallate pair, lauryl gallate displayed a higher antioxidant activity than gallic acid (Figure 4), which represents the highlighted region in Figure 4b. This trend is similar to that of ascorbic acid/ascorbyl palmitate and not in agreement with what the polor paradox predicts.

Figure 5 shows the antioxidant activity of EGCG and its tetrastearate. The nonpolar EGCG-tetrastearate was a better antioxidant than the polar EGCG below the concentration of 0.08 μmol/g, whereas at higher concentrations EGCG was more effective than its nonpolar ester. This reflects the highlighted region in Figure 5b. Moreover, it appears that 0.08 μmol/g may be the critical concentration for this antioxidant pair, and the polar paradox is valid only at concentrations higher than 0.08 μmol/g.

In conclusion, the results from this preliminary study confirm the hypothesis that polar paradox experiences limitations in bulk oil and its validity is also dependent on the concentration of the antioxidants employed. A critical concentration exists at which antioxidants behave differently in response to polarity, as illustrated by the model in Figure 1. However, the results in this study are based on conjugated dienes alone; further investigation using more comprehensive secondary oxidation products as indicators and selecting broader or narrower ranges of antioxidants may be helpful to fully unravel the behavior of antioxidants in various media.

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