Food Chemistry 206 (2016) 119–123

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

"Cut-off" effect of antioxidants and/or probes of variable lipophilicity in microheterogeneous media

Carolina Aliaga [a,b,*], Amaia López de Arbina [a], Marcos Caroli Rezende [a]

[a] Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 40 Correo 33, Santiago de Chile, Chile
[b] Centro para el Desarrollo de la Nanociencia y la Nanotecnología, CEDENNA, Chile

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form 9 February 2016
Accepted 8 March 2016
Available online 9 March 2016

Keywords:
Hydrophobicity
4-AlkanoyloxyTEMPO
Micelle
EPR
Antioxidant activity
Ascorbate
BHT
α-Tocopherol
Trolox

ABSTRACT

The activities of two hydrophilic (ascorbic acid and Trolox) and two hydrophobic (α-tocopherol and BHT) antioxidants were measured by reaction with a series of 4-alkanoyloxyTEMPO radical probes **1** in buffered (pH 7), aqueous, micellar solutions of reduced Triton-X 100. In all cases, a cut-off effect was observed, in line with previous observations of the same effect for the partitioning of probe series **1** in this medium. These results support an interpretation of the cut-off effect in food emulsions, based on the "amphiphobic" nature of either the antioxidants or probes: competition between two molecular moieties, for the micellar hydrophobic core, tends to expose a reacting fragment differently to a more hydrophilic microenvironment, as the probe or antioxidant hydrophobicity increases.

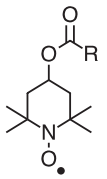
© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The assessment of the antioxidant activities in emulsions has been a subject of general interest for various chemists dedicated to food research. Considering the very frequent occurrence of microheterogeneous media in food extracts, the question has been raised as to how meaningful literature data are, when the partitioning of either probe or antioxidant in such media is not taken into account (Aliaga, Rezende, & Arenas, 2009). It is shown that, in the presence of a micelle, the antioxidant activity of different phenols, measured with a tetramethylpiperidinyloxy (TEMPO) probe, differed widely, according to their partitioning in the micellar environment and the relative lipophilicity of the radical probe. For an accurate determination of the partitioning of radical probes of variable lipophilicity in microheterogeneous media, a method has been developed, based on the measurement of the *g*-value of TEMPO derivatives, that yielded information about their partitioning and location inside the micelle (Aliaga, Torres, & Silva, 2012). By systematically varying the lipophilicity of a set of TEMPO derivatives in an aqueous micellar solution, it could also be shown that their partitioning and insertion did not increase monotonically with the probe lipophilicity, but reflected a more subtle orientation effect inside the hydrophobic microenvironment (Aliaga et al., 2016). As a result, it was proposed that a new interpretation for the so-called "cut-off" effect, well-known to many food chemists (Laguerre et al., 2009; Losada Barreiro, Bravo-Díaz, Paiva-Martins, & Romsted, 2013), as reflecting, in addition to their lipophilicity, the location and orientation of a probe or antioxidant in a hydrophobic microenvironment (Aliaga et al., 2016). This paradoxical effect, first identified by Porter (Porter, Black, & Drolet, 1989), arises from experimental evidence that non-polar antioxidants are frequently more active in emulsified media than their polar homologues (Costa, Losada-Barreiro, Paiva-Martins, Bravo-Díaz, & Romsted, 2015). Our finding that, in aqueous solutions of Triton-X 100, the partition coefficients of the TEMPO derivatives **1** (Scheme 1) attained maximum values for R = C6–C8, decreasing then with the increasing lipophilicity of the probe, could be interpreted with the aid of molecular simulations (Aliaga et al., 2016). According to this view, the cut-off effect observed, in emulsions, results from the "amphiphobic" nature of a series of probes and/or antioxidants. "Amphiphobic" molecules with two moieties that compete for the hydrophobic core of a micelle tend to orient themselves and to expose their reacting fragment differently to a more hydrophilic microenvironment, as their total hydrophobicity

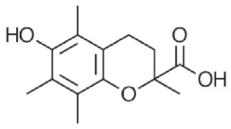
* Corresponding author at: Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 40 Correo 33, Santiago de Chile, Chile
E-mail address: carolina.aliaga@usach.cl (C. Aliaga).

http://dx.doi.org/10.1016/j.foodchem.2016.03.024
0308-8146/© 2016 Elsevier Ltd. All rights reserved.

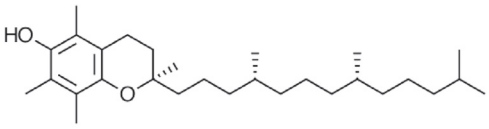


1

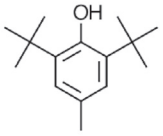
R = CH_3 (**a**); n-C_3H_7 (**b**); n-C_5H_{11}(**c**); n-C_7H_{15} (**d**); n-C_9H_{19} (**e**); n-$C_{11}H_{23}$ (**f**)



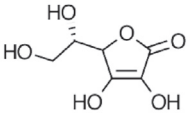
2



3



4



5

Scheme 1. Structure of the 4-alkanoyloxyTEMPO probes **1a**–**f** and the antioxidants employed in the present work: Trolox (**2**), α-tocopherol (**3**), BHT (**4**) and ascorbic acid (**5**).

increases. To substantiate this view, the activities of various hydrophilic or hydrophobic antioxidants in a microheterogeneous environment, as measured by probes **1**, should also reflect this previously observed partitioning "cut-off".

A recent work, employing the same series of TEMPO derivatives for the assessment of the antioxidant activity of the ascorbate anion in tetradecane-in-water microemulsions, seemed to refute the above expectations (Leong et al., 2015). The authors observed that, in this emulsion, reduction rates by the hydrophilic ascorbate decreased with the increased lipophilicity of the probes, and no "cut-off" effect was observed. (Leong et al., 2015).

Therefore, it was decided to investigate the relative reactivities of probes **1** in a micellar medium, *vis-à-vis*, three different phenolic antioxidants of variable lipophilicity: hydrophilic Trolox **2**, and hydrophobic α-tocopherol (**3**) and butylated hydroxytoluene, BHT (**4**), shown in Scheme 1. In addition, it was decided to include in our set of antioxidants the hydrophilic ascorbic acid **5**, in order to confirm the absence of a "cut-off" effect in micellar solutions of **1**.

2. Materials and methods

2.1. General

α-Tocopherol, Trolox, BHT and ascorbic acid were purchased from Sigma Aldrich. The TEMPO derivatives **1a**–**f** were prepared as previously described (Aliaga et al., 2016).

EPR spectra were recorded on a Bruker EMX-1572 operating at X-band (9.0–9.9 GHz), at 21 ± 1 °C.

2.2. EPR measurements

Sample preparations were the same as those previously described (Aliaga et al., 2016).

Control solutions of probes **1a**–**f** (50 μM) in buffered micellar solutions (pH 7) of reduced Triton-X100 (11.0 wt%, 20 mM) (Sigma–Aldrich) were prepared above the critical micelle concentration, to ensure complete dissolution of the probes in the micellar media. An aliquot (10 μl) of a methanolic solution of probe **1** (1 mM) was then added to a buffered (pH 7) solution of the appropriate antioxidant, in the presence of reduced Triton-X100 (11.0 wt %, 20 mM) and the resulting 200 μl solution was thoroughly stirred before signal recording in the EPR cavity. The final concentration of the probe in these solutions was approximately 50 μM. The corresponding final concentration of the antioxidant at the beginning of each run was considerably larger (10 mM), to ensure pseudo-first-order conditions. Spectra were measured 2 min after addition of the probe to the antioxidant solution and compared with measurements after equilibrium was reached. The following EPR parameters were kept constant in all experiments: microwave power, 1 mW; modulation amplitude, 5 G; time constant, 10.24 ms; and conversion time, 40.96 ms.

3. Results and discussion

The reaction of the TEMPO derivatives **1a**–**f** with phenols **2**, **3** or **4** proceeded at pH 7, with all reagents partitioned between the aqueous and micellar phases. At this pH, all phenols were protonated, since their pKa values were much higher than 7: Trolox (11.92) (Alberto, Russo, Grand, & Galano, 2013), α-tocopherol in micelles (11.0–14.0) (Drummond & Grieser, 1985) and BHT (12.23) (Fereidoon, 2015). The mechanism of these reactions was a hydrogen-abstraction process by the TEMPO fragment, leading to a final equilibrium between the reacted and unreacted probe (Scheme 2).

The reactivity of these TEMPO derivatives could, therefore, be obtained by their relative consumption in the presence of the antioxidant after equilibrium. Under the pseudo-first-order conditions employed in the present work, these equilibria for ascorbate and Trolox were attained 4 min after recording the initial radical EPR spectrum. For α-tocopherol and BHT, the equilibrium was reached after 20 min.

Fig. 1 reproduces the relative reactivities of TEMPO esters **1a**–**f** *vis-à-vis* hydrophilic Trolox **2**, α-tocopherol **3** and BHT **4**.

Download English Version:

https://daneshyari.com/en/article/1184236

Download Persian Version:

https://daneshyari.com/article/1184236

Daneshyari.com