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Antioxidant activity of alkyl gallates and glycosyl alkyl gallates in fish oil in water emulsions: Relevance of their surface active properties and of the type of emulsifier

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ABSTRACT

The antioxidant activity of gallic acid and a series of alkyl gallates (C4–C18) and glycosylated alkyl gallates (C4–C18) on fish oil-in-water emulsions was studied. Three types of emulsifiers, lecithin, Tween-20 and sodium dodecyl sulphate (SDS) were tested. A nonlinear behavior of the antioxidant activity of alkyl gallates when increasing alkyl chain length was observed for emulsions prepared with lecithin. Medium-size alkyl gallates (C6–C12) were the best antioxidants. In contrast, for emulsions prepared with Tween-20, the antioxidants seem to follow the polar paradox. Glucosyl alkyl gallates were shown previously to be better surfactants than alkyl gallates. Nevertheless, they exhibited a worse antioxidant capacity than their corresponding alkyl gallates, in emulsions prepared with lecithin or Tween-20, indicating the greater relevance of having three OH groups at the polar head in comparison with having improved surfactant properties but just a di-ortho phenolic structure in the antioxidant.

1. Introduction

Polyunsaturated fatty acids (PUFA) are major components in fish oil and are known to be highly beneficial for human health (Bang, Dyerberg, & Nielsen, 1971; Dyerberg, Bang, Stoffersen, Moncada, & Vane, 1978; Tziomalos, Athyros, & Mikhailidis, 2007). This aspect has made them very attractive for the food, nutraceutical and cosmetics industries. However, the use of marine lipids is quite challenging due to the presence of highly oxidizable unsaturated fatty acids (Hsieh & Kinsella, 1989). Lipid oxidation becomes an even larger problem when they are part of dispersed lipid systems such as oil-in-water emulsions. This type of matrix is characterized by a large interfacial area and it is at this exact location where lipid oxidation has been proposed to start before propagating to the rest of the oil phase (Frankel, 1998; McClements & Decker, 2000).

Among the different strategies used to retard or inhibit lipid oxidation, the addition of antioxidants is one of the most employed approaches. Understanding the efficiency of antioxidants in inhibiting oxidation is a relevant subject for designing and preparing better antioxidants. Thus, these compounds will help fish oil containing products to extend their shelf life and maintaining their nutritional and health-related properties.

A long time standing theory to predict the antioxidant efficiency on different oil matrices has been the "polar paradox" proposed by Porter (1980) and Porter, Black, and Drolet (1989) which states that polar antioxidants are more effective in bulk oils, whereas lipophilic antioxidants display better antioxidant activity in emulsified systems. Frankel, Huang, Kanner, and German (1994) contributed to explanation of these experimental findings with the concept of interfacial oxidation. They proposed that the differences observed may be explained by the affinity of polar antioxidants for the air–oil interface in bulk oils due to their low solubility in oil, whereas lipophilic antioxidants would prefer to locate at the oil–water interphase in emulsions.

Several research groups (Chaiyasit, McClements, & Decker, 2005; Kikuzaki, Hisamoto, Hirose, Akiyama, & Taniguchi, 2002; Laguerre et al., 2009, 2010; Medina, Lois, Alcántara, Lucas, & Morales, 2009; Stöckmann, Schwarz, & Huynh-Ba, 2000; Sørensen et al., 2008, 2011; Torres de Pinedo, Peñalver, & Morales, 2007a; Torres de Pinedo, Peñalver, Pérez-Victoria, Rondón, & Morales, 2007b; Yuji et al., 2007) have found different

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examples that question the validity of the polar paradox. We found that small structural changes at phenolipids and other phenolic-based antioxidants affecting their polarity can display different antioxidant activity in bulk oils than that predicted by the polar paradox (Torres de Pinedo, Peñalver, et al., 2007a, 2007b). Recently, Zhong and Shahidi (2012) have reported a preliminary study with several polar and nonpolar representative antioxidants in bulk oil where concentration seems to play a critical role and therefore the polar paradox is applicable over certain concentration ranges (Shahidi & Zhong, 2011).

In emulsions and liposomes, different authors have reported that an increase in hydrophobicity was not always advantageous for antioxidant effectiveness (Kikuzaki et al., 2002; Medina et al., 2009; Stöckmann et al., 2000; Sørensen, de Diego, Petersen, Nielsen, & Yang, 2010; Yuji et al., 2007). In fact, a parabolic (or cut-off) effect on antioxidant activity was noticed when increasing the length of the homologous series of lipophilic alkyl esters of chlorogenic and rosmarinic acids (Laguerre et al., 2009, 2010). Consequently, medium-size chains yielded the best antioxidant capacity in emulsions, in contrast with the prediction by the polar paradox.

Different explanations have been proposed for this parabolic effect on antioxidant efficiency in emulsions such as partitioning factors of antioxidants in emulsified systems (Laguerre et al., 2009), reduced mobility (Fendler, 1982; Laguerre et al., 2013; Losada-Barreiro, Sanchez-Paz, & Bravo-Diaz, 2013), internalization (Laguerre et al., 2013), self-aggregation of phenolipids with very long alkyl chains due to their hydrophobicity and molecular size (Laguerre et al., 2010, 2013; Panya et al., 2012) and surface active properties of the phenolipid antioxidants (Heins, McPhail, Sokolowski, Stöckmann, & Schwarz, 2007; Lucas et al., 2010; Yuji et al., 2007).

Our objective in this work was to investigate the efficiency of antioxidants in oil-in-water emulsions by examining the relevance of the surface active properties and the molecular interactions between the phenolipid antioxidants and the emulsifier. To do so, we designed and prepared a series of alkyl gallate derivatives containing carbohydrates on the phenolic moiety and examined them as inhibitors of the oxidation of highly oxidation susceptible fish lipids when contained in oil-in-water emulsions (Fig. 1). We have recently shown that by adding a sugar to alkyl gallates at their phenolic structure, the corresponding glycosyl alkyl gallates become better surfactants. The idea was to check the antioxidant capacity of these new molecules with improved surfactant efficiency but containing just a di-ortho phenolic structure (in comparison with their parent compounds containing three phenolic OH's). Oxidation experiments in oil-in-water emulsions have been carried out using lecithin, Tween-20 and SDS as emulsifiers. The rate of oxidation was monitored by the formation of lipid oxidation products during controlled sample storage.

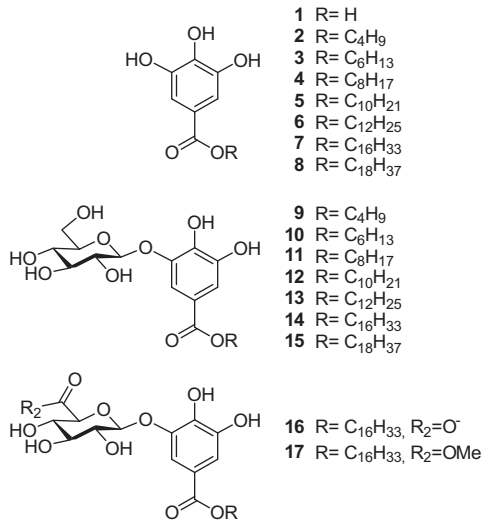

Fig. 1. Chemical structures of gallic acid **1**, alkyl gallates **2**–**8**, glucosyl alkyl gallates **9**–**15**, and glucuronosyl alkyl gallate **16** and glucuronosyl methyl ester hexadecyl gallate **17**.

2. Materials and methods

2.1. Materials

Cod (*Gadus morhua*) liver oil contained 40.6% of ω-3 PUFA's (3.7% of 18:3ω3; 1.3% of 20:4ω3; 14.9% of 20:5ω3; 2.8% of 22:5ω3 and 17.9% of 22:6ω3) was purchased from Fluka (New-Ulm, Switzerland). It showed a standard quality as tested by the absence of rancid off-flavors as well as low values of hexanal (less than 0.01 ppm), 1-penten-3-ol or pentanal (both lower than 0.001 ppm) (Iglesias, Lois, & Medina, 2007). Its peroxide and anisidine values were 3.92 ± 0.35 milliequivalents oxygen/kg oil (Chapman & Mackay, 1949) and 10.32 ± 0.56 (AOCS, 2011 Method Cd 18-90), respectively.

L-α-phosphatidylcholine (Soybean lecithin, Sigma, St. Louis, MO, USA), Tween-20 (Sigma) and SDS (Sigma) were used as surfactant in oil-in-water emulsions. Soybean lecithin used was essentially a crude organic extract of egg yolk which contains not less than 60% phosphatidylcholine. The remaining 40% consists of mostly phosphatidylethanolamine plus other phospholipids as well as traces of triacylglycerols and cholesterol. Its peroxide and anisidine values were 6.78 ± 0.14 milliequivalents oxygen/kg oil (Chapman & Mackay, 1949) and 0.85 ± 0.02 (AOCS, 2011 Method Cd 18-90), respectively. Gallic acid (Sigma) was used as control since is the basic unit of the different phenolipids. Butyl gallate, hexyl gallate, octyl gallate, dodecyl gallate, hexadecyl gallate and octadecyl gallate were purchased from TCI Europe. N.V (Boerenveldseweg, Zwijndrecht, Belgium). Decyl gallate was prepared as described previously (Maldonado et al., 2011). All chemicals and solvents used were either analytical or HPLC grade (Ridel-Haën, Seelze, Germany). Water was purified through a Millipore-Q plus (Millipore Corp., Bedford, MA, USA).

2.2. Synthesis of glucosyl- and glucuronosyl alkyl gallates

The new phenolipids were prepared from the corresponding alkyl gallates as described previously (Maldonado et al., 2011) (see Fig. 1 for structures). Glucuronosyl methyl ester hexadecyl gallate, compound **17**, was synthesized as follows: acetyl protected glucuronosyl methyl ester hexadecyl gallate was dissolved in methanol (2 mL for each 100 mg) and Na_2CO_3 (0.3 eq.) was then added. The reaction mixture was stirred for 1 h and when starting material had disappeared, Amberlite IR-120 was then added until pH = 7. The reaction mixture was then filtered and solvents removed to afford compound **17** in high yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (s, 1 H, H_{arom}), 7.18 (s, 1 H, H_{arom}), 4.81 (d, 1H, J = 7.32, H-1), 4.49 (t, 2H, CH_2), 3.96 (d, 1H, J = 9.6 Hz, H-5), 3.71 (s, 3H, MeO), 3.60–3.45 (m, 3H, H-2, H-3, H-4), 1.67-1.63 (m, 2H, CH_2), 1.34-1.19 (m, 26 H, 13 × CH_2), 0.82 (t, 3H, J = 7.5 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 166.6, 145.6, 145.1, 140.3, 120.6, 112.0, 110.4, 102.9, 75.4, 73.1, 71.5, 64.6, 51.6, 31.7, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 28.4, 25.7, 22.4, 13.1. MS (ES^+) Calcd. for $C_{30}H_{48}NaO_{11}$ (M−H) 583.3, found: 583.6. All compounds prepared showed 95% purity or higher by HPLC.