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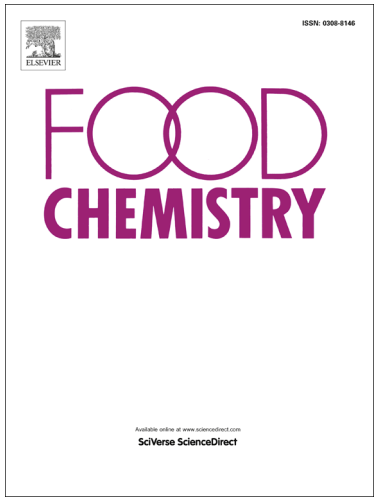

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Antioxidant Activity of Alkyl Gallates and Glycosyl Alkyl Gallates in Fish oil in Water Emulsions: Relevance of their Surface Active Properties and of the type of emulsifier

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Running Title. Antioxidant activity of alkyl gallates and derivatives in fish oil in water emulsions

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Abstract

The antioxidant activity of gallic acid and a series of alkyl gallates (C4 to C18) and glycosylated alkyl gallates (C4 to 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 behaviour 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.

Highlights

- Alkyl gallates exhibited good antioxidant (AO) activity in fish oil-in-water emulsions.
- The type of emulsifier affects their AO activity when increasing alkyl chain length.
- Glucosyl alkyl gallates showed to be better surfactants than alkyl gallates.
- Glucosyl alkyl gallates presented worse AO activity than alkyl gallates.
- Maintenance of the three OH groups at the antioxidant is crucial for AO activity.

Keywords: Lipid oxidation; gallic acid; alkyl gallates, glycosylation, oil-in-water emulsions, antioxidant, surfactant;

INTRODUCTION

Polyunsaturated fatty acids (PUFA) are major components in fish oil and are known to be highly beneficial for human health (Bang *et al.*, 1971; Dyerberg *et al.*, 1978; Tziomalos *et al.*, 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 *et al.*, 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 *et al.*, 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 *et al.* (1989) which states that polar antioxidants are more effective in bulk oils, whereas lipophilic antioxidants display better antioxidant activity in emulsified systems. Frankel *et al.* (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 *et al.*, 2005; Kikuzaki *et al.*, 2002; Laguerre *et al.*, 2009; Laguerre *et al.*, 2010; Medina *et al.*, 2009; Sørensen *et al.*, 2008; Sørensen *et al.*, 2011; Stöckmann *et al.*, 2000; Torres de Pinedo *et al.*, 2007a; Torres de Pinedo *et al.*, 2007b; Yuji *et al.*, 2007) have found

different 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 *et al.*, 2007a; Torres de Pinedo *et al.*, 2007b). Recently, Zhong *et al.* (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 *et al.*, 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; Sørensen *et al.*, 2010; Stöckmann *et al.*, 2000; 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.*, 2012; Losada-Barreiro *et al.*, 2013), internalization (Laguerre *et al.*, 2012), self-aggregation of phenolipids with very long alkyl chains due to their hydrophobicity and molecular size (Laguerre *et al.*, 2010; 2012; Panya *et al.*, 2012) and surface active properties of the phenolipid antioxidants (Heins *et al.*, 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 (Figure 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.

MATERIALS AND METHODS

Materials. Cod (*Gadus morhua*) liver oil contained 40.6 % of -3 PUFA's (3.7% of 18:3 3; 1.3% of 20:4 3; 14.9% of 20:5 3; 2.8% of 22:5 3 and 17.9% of 22:6 3) was purchased from Fluka (New-Ulm, Switzerland). It showed a standard quality as tested by the absence of rancid off-flavours 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 *et al.*, 2007). Its peroxide and anisidine values were 3.92 ± 0.35 milliequivalents oxygen/ kg oil (Chapman *et al.*, 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 *et al.*, 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).

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 Figure 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, 13xCH_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.

Preparation of oil-in-water emulsions and thermal oxidation experiments. Cod liver oil-in-water emulsions containing the emulsifier (1% lecithin, 2 % Tween-20 and 1% SDS) and 10% fish oil were prepared in water, as previously described by Huang *et al.* (1996b). Briefly, cod liver oil was emulsified in water using lecithin, Tween-20 or SDS as emulsifiers, and sonicated at high power (Ultrason Fungilab, 30 KHz ± 5%) for 10 min in a cold glass container. Previous studies in our laboratory showed that these are the most adequate concentrations of each emulsifier to get a stable emulsion during the whole study. Prepared phenolipids were added in methanol solutions into screw-capped 50-mL Erlenmeyer flasks and then, methanol was removed under a stream of nitrogen before addition of oil-in-water emulsions (3 g). The concentration of each phenolipid in the emulsion was 0.1 mmol/kg. Samples were subsequently sonicated for 5 min for a total dispersion of antioxidants. Control samples have no antioxidant added. The oxidative stability of emulsions was monitored during storage at two different

144 temperatures 45 ºC and 30º C by sensory analysis and measuring the formation of conjugated diene and
145 triene hydroperoxides. The set of experiments including different phenolipids that share the hexadecyl
146 alkyl chains was carried out at 50 ºC to accelerate sample oxidation. Triplicate samples were prepared
147 and oxidized.

148 **Sensory analysis.** Sensory analysis was evaluated by an expert panel formed by four trained specialists
149 in descriptive analysis of fishy off-flavours, in a room designed for that purpose. Samples were placed at
150 room temperature during 10 minutes before analysis. Three categories were ranked: no rancidity **(A)**,
151 incipient rancidity **(B)**, and rancid **(C)**.

152

153 **Conjugated diene and triene hydroperoxides.** Fifty microliters of emulsion (49 mg) were dispersed in
154 5 mL of ethanol and then diluted to a measurable absorbance when it was necessary. The absorbance
155 was measured at 234 nm for dienes and at 268 nm for trienes (UV-Vis Spectrophotometer, Perkin
156 Elmer, Waltham, MA, USA). The results were expressed as millimol of hydroperoxides per kilogram of
157 oil (mmol/kg oil) as describes previously (Huang *et al.*, 1996a).

158 % Inhibition was determined according to equation:

159 % INHIBITION = ((C-S)/C) *100)

160 Where C was the increment in the oxidation product formed in control and S was the increment in the
161 oxidation product formed in sample, both expressed as mmol / kg oil.

162 **Statistical Analysis**. Each sample type (antioxidant) was replicated in two independent storage
163 experiments (n=2) using different batches of oil-in-water emulsions. Triplicate samples were prepared
164 for the each of those experiments. An average value of the replicate analyses was used in calculations of
165 sample variation and significance testing. The data were compared by one-way analysis of variance and
166 the means were compared by a least squares difference method *(*Sokal *et al., 1994).* Significance was
167 declared at $p < 0.01$. Correlations between the propagation rates of lipid oxidation products and the
168 physicochemical properties of phenolics were determined by Pearson coefficients. Statistical analyses
169 were performed with the software *Statistica*.

RESULTS

Preparation of glucosyl- and glucuronosyl alkyl gallates. These compounds have been synthesized from the corresponding alkyl gallates **2**-**8** (Maldonado *et al.*, 2011) (see Figure 1 for structures). Briefly, two contiguous hydroxyl groups of the alkyl gallates were protected via isopropylidene formation in moderate yields (43-60%). Next, the remaining OH group was glycosylated with the acetyl-protected glucosyl or glucuronosyl methyl ester trichloroacetimidate donors (yields 67-93 and 42-63%, respectively). Finally, treatment with trifluoroacetic acid to remove the acetal group (yields 53-83% for the glucose series and 62-81% for the glucuronosyl series) followed by basic hydrolysis gave glucosyl alkyl gallates **9**-**15** (yields 75-99%) and glucuronosyl alkyl gallate **16** (yield 79%). Compound **17** was obtained by shortening the reaction time during the basic hydrolysis of the corresponding acetyl protected glucuronosyl methyl ester hexadecyl gallate. All compounds were purified by flash chromatography using silica gel as stationary phase. Further details on the synthesis and purification can be found in Maldonado *et al.* (2011).

Inhibition of lipid oxidation by alkyl gallates 2-8. Antioxidant activity of alkyl gallates and gallic acid in fish oil-in-water emulsions was tested in thermal oxidation samples supplemented with concentrations of 0.1 mmol/kg of each compound. The temperature and time of the experiment varies depending on the concentration and efficiency of the antioxidants and the emulsifier used in the oxidation experiment. The oxidation experiments were first run during 10 days at 45 ºC using lecithin as emulsifier. According to sensory assessment (Table S1, supplementary data) the best results were obtained with hexyl gallate (**3**) which kept the emulsion stable until day 10. Control samples developed incipient rancidity by the 4th day. Samples with the rest of alkyl gallates showed a good quality until, at least, the sixth day. These results were verified by chemical analysis of conjugated diene and triene hydroperoxides (Figure S1, supplementary data). Results on the percentage of inhibition on the

196 formation of conjugated diene and triene hydroperoxides are shown in Table 1. All alkyl gallate
197 derivatives were considerably effective to inhibit the formation of conjugated diene and triene
198 hydroperoxides. The antioxidant efficiency order was found to be: hexyl gallate ~ dodecyl gallate >
199 octyl gallate ~ decyl gallate ~ hexadecyl gallate > butyl gallate > octadecyl gallate >> gallic acid.
200 These results seem to disagree with the rules predicted by the polar paradox since the two more
201 hydrophobic compounds, hexadecyl and octadecyl gallates (**7** and **8**, respectively), display worse
202 antioxidant capacity than some medium size, less polar derivatives such as hexyl or octyl gallate (**3** and
203 **4**, respectively).

204 When the emulsion was prepared with Tween-20 or SDS as emulsifiers and the experiments
205 were carried out at 45ºC, there was a notable increment of the rate of oxidation. As consequence of this
206 oxidation rate and the lack of induction period, it was difficult to study the antioxidant behavior of the
207 target compounds and therefore, identify differences on the antioxidant efficiency among them (data not
208 shown). Then, all the following experiments with Tween-20 or SDS were carried out at 30ºC. It is
209 important to comment that lecithin is a known antioxidant compound (Evans, 1935; Feigenbaum, 1946;
210 Judde *et al.*, 2003) whereas Tween-20 and SDS are emulsifiers without any known antioxidant
211 properties.

212 When the oxidation experiments were run using Tween-20 as emulsifier at 30 ºC for 5 days,
213 conjugated diene and triene hydroperoxide data showed that most alkyl gallates were quite effective
214 antioxidants (Table 1 and Figure S2, supplementary data). Only butyl gallate showed medium
215 antioxidant efficiency and in the case of gallic acid a prooxidant behaviour was observed. The
216 antioxidant efficiency order showed some differences compared to the experiment with lecithin as
217 emulsifier: dodecyl gallate ~ hexadecyl gallate ~ octadecyl gallate > octyl gallate ~ hexyl gallate >
218 decyl gallate >> butyl gallate >> gallic acid. In this case, the highest antioxidant efficiency is observed
219 for the more hydrophobic alkyl gallates **6**-**8**, as it would be predicted by the "polar paradox". Sensory
220 assessment (Table S2A, supplementary data) showed that gallic acid developed incipient rancidity by

the first day, whereas dodecyl gallate, hexadecyl gallate and octadecyl gallate showed the best results and did not show rancidity until day 5. Sensory scores agreed with chemical analysis results.

In emulsions prepared with SDS as emulsifier (Figure S3, supplementary data), all compounds showed a prooxidant behaviour (Table S3) developing a rancid off-flavors by the second day of storage (Table S4A).

Inhibition of lipid oxidation by glucosyl alkyl gallates 9-15. The effect of the addition of a glucose unit at the phenolic ring of alkyl gallates on the antioxidant activity in emulsions was examined next. Thermal oxidation experiments in fish oil-in-water emulsions were carried out in samples supplemented with each phenolic derivative (0.1 mmol/kg). Emulsions were prepared first using lecithin as emulsifier and oxidation experiments were run during 8 days at 45 ºC. For direct comparison the corresponding alkyl gallates were also added to the experiment.

According to sensory assessment (Table S5), control samples and all glucosyl alkyl gallates were kept stable until day 4. After that, a rancid odour was detected. Sensory evaluations were verified by chemical analysis of conjugated diene and triene hydroperoxides (Table S6). All glucosyl alkyl gallate derivatives **9**-**15** showed very little efficiency to inhibit the formation of conjugated diene and triene hydroperoxides (Figure S4, supplementary data). Only glucosyl hexyl gallate **10** displayed a very limited antioxidant activity. The presence of the third hydroxyl group at the phenolic unit seems to be crucial for the antioxidant capacity.

We decided to perform the same experiment under less drastic conditions (30 ºC, see Table 2 and Figure S5, supplementary data) trying to differentiate more clearly among this series of antioxidants. In this case, medium size glucosyl alkyl gallates (**9**-**12**) showed reasonable antioxidant capacity after 8 days with glucosyl butyl gallate **9** being the best antioxidant of this series according to sensory scores (Table S7B, supplementary data) and conjugated diene hydroperoxides formation. Similarly to the oxidation experiment in emulsions prepared with lecithin containing alkyl gallates, the most hydrophobic compounds (**13**-**15**) were worse antioxidants than some of the more polar compounds

of the series (**9**-**12**).Then, we carried out the oxidation experiments in emulsions prepared with Tween-20 as emulsifier (30 ºC for 5 days). Conjugated diene and triene hydroperoxide data (Table 2**,** see also Figure S2, supplementary data) showed quite different results from those obtained in emulsions prepared with lecithin. Here, glucosyl butyl gallate **9** displayed the worst antioxidant activity of the series, followed by glucosyl decyl gallate **12.** The rest of derivatives were better antioxidants, with the most hydrophobic glucosyl octadecyl gallate **15** being the best antioxidant of this series. Again, sensory analysis agreed with these results (Table S2B) showing the worst quality for glucosyl butyl gallate **9** by the second day.

Experiments with SDS as emulsifiers were carried out at 30 ºC during 3 days (Table S8 and Figure S3, supplementary data). Only glucosyl decyl gallate showed moderate antioxidant behaviour inhibiting the development of rancidity up to second day (Table S4B). The rest of compounds showed a prooxidant behaviour, the same result observed for the alkyl gallates series.

Inhibition of lipid oxidation by glucosyl hexadecyl gallate 14, glucuronosyl hexadecyl gallates 16 and glucuronosyl methyl ester hexadecyl gallate 17. As a final experiment we compared the antioxidant efficiency in fish oil-in-water emulsions of three different modifications on the phenolic ring of hexadecyl gallate with the unmodified hexadecyl gallate **7**. We included glucosyl, glucuronosyl and glucuronosyl methyl ester hexadecyl gallates, compounds **14, 16** and **17**. Compound **16** behaves as a surfactant (surface tension changes with concentration in aqueous solution) whereas **14** and **17** do not. Once again, thermal oxidation samples were supplemented with concentrations of 0.1 mmol/kg of each antioxidant. The oxidation experiments were run at 50 ºC during 4 days first on emulsions prepared with lecithin. Octyl gallate **4** was used as positive control.

The results on the percentage of inhibition on the formation of conjugated diene and triene hydroperoxides are shown in Table 3 (see also Figure S6, supplementary data). All three phenolic ring modified hexadecyl gallates (**14**, **16** and **17**) showed a clear lower antioxidant efficiency compared to hexadecyl gallate **7**. Among them, no differences could be observed between compounds **14** and **16**.

Compound **17** was the less active antioxidant in this system. Chemical results agreed with sensory assessment (Table S9, supplementary data).

When the thermal oxidation experiment was carried on emulsions prepared with Tween-20 as emulsifier at 30ºC, the results for the phenolic ring modified alkyl gallates **14**, **16** and **17** were according with those previously described for lecithin since they showed less antioxidant efficiency than the original hexadecyl gallate **7** that showed a notable antioxidant efficacy. (Figure S2**,** supplementary data). The type of unit attached to the phenolic ring has a small influence on the antioxidant activity of these derivatives. Among the gallates with a phenolic ring substituent **14**, **16** and **17**, compound **16** showed the highest efficiency. Sensory score agreed with these results (Table S2B, supplementary data).

Again, a prooxidant activity of these compounds was observed when emulsion was prepared with SDS as emulsifier at 30 ºC (Figure S3, supplementary data) showing a rancid off-flavor by the first day (Table S4, supplementary data).

DISCUSSION

Alkyl gallates as antioxidants for emulsions have been studied previously by different groups. Porter *et al.* (1989) examined gallic acid and alkyl gallates up to twelve carbons length (dodecyl gallate) in dry vegetable oil-in-water emulsions using lecithin as emulsifier. When they plotted antioxidant effectiveness against the R_f measured on silica TLC plates (that gives a rough measure of polarity), the authors found a general linear trend where nonpolar antioxidants were more effective in dispersed lipid emulsions. In fact, this has been considered a clear example of antioxidants that support the "polar paradox".

We have plotted the percentage of oxidation inhibition found in a fish oil-in-water emulsion against the alkyl chain length for each antioxidant of this alkyl gallate series (Figure 2A) and have found a parabolic behaviour when lecithin was used as emulsifier and a non-linear hyperbolic curve when Tween-20 was used as emulsifier. When SDS was used as emulsifier, only glucosyl decyl gallate

showed moderate antioxidant behaviour. The rest of compounds showed a clear prooxidant action. Our results with emulsions using lecithin as emulsifier seem to disagree with the rules predicted by the polar paradox. The short series of alkyl gallates used by Porter and colleagues in their experiments may be the reason for the discrepancies with our results. In contrast, our results with emulsions using Tween-20 as emulsifier seem to fit better with the polar paradox since the more hydrophobic compounds display better antioxidant efficiency in emulsions. In fact, a decrease in the percentage of oxidation inhibition is not observed for hexadecyl gallate **7** or octadecyl gallate **8** on emulsions prepared with Tween-20 but it is observed on emulsions prepared with lecithin.

Several studies of antioxidant efficiency of phenolipids in emulsions have been reported. Laguerre *et al.* (2009; 2010) found a parabolic behaviour or cut-off effect on a series of chlorogenate alkyl esters and rosmarinate alkyl esters where the maximum antioxidant efficiency in an oil-in-water system was displayed by medium-size alkyl derivatives (dodecyl and octyl, respectively). Acylation of hydroxytyrosol with medium-size alkyl chains (octanoic acid) also displayed higher antioxidant activity than hydroxytyrosol itself or hydroxytyrosol fatty acid esters with longer alkyl chains in fish oil-in-water emulsions (Medina *et al.*, 2009).

Several explanations have been proposed for this type of behaviour. Since location of the antioxidants at the oil-water interphase is considered crucial to obtain good antioxidant activity in emulsions (Heins *et al.*, 2007) it makes sense that the partitioning behaviour of the antioxidants between the different phases could be key to explain the parabolic effect. However, Laguerre *et al.* (2009) did not observe a good correlation with partitioning and proposed that long-chain phenolipids could be involved in the formation of micelles or other aggregates and therefore not properly placed at the emulsion interphase. The decrease in mobility due to the increase in molecular size for long-chain lipophilic antioxidants has also been mentioned as a possible reason for the parabolic effect observed (Fendler, 1982). Once again, this decrease in diffusion of the antioxidants may hinder the proper location of the antioxidants at the interphase.

Recently, we have shown that phenolipids such as hydroxytyrosol fatty acid esters possess surfactant properties (Lucas *et al.*, 2010) and have proposed that the more effective surfactants would locate preferentially at the oil-water interface in the emulsions inhibiting lipid oxidation more efficiently. In a previous work we measured the surfactant properties in aqueous solutions of the alkyl gallates **2**-**8** used in this study (see Table S10) (Maldonado *et al.*, 2011). When we plot the surfactant effectiveness versus the length of the alkyl chain for each of the alkyl gallates that display surfactant properties, we observe that the data easily fit a parabolic line (Figure S7). In fact, the best surfactants are medium-size alkyl gallates that also display the best antioxidant efficiency in oil-in-water emulsions when lecithin is used as emulsifier. However, since this surfactant property is linked to the structure of the antioxidant, there is not such a correlation with the antioxidant capacity observed for the alkyl gallates in emulsions prepared with Tween-20 as emulsifier where medium-size and long-size alkyl gallates show similar antioxidant efficiency.

One could argue that the antioxidant behaviour of the phenolipids is ruled in a different way for each specific emulsifier used to prepare the emulsions. This is probably not the case since Panya *et al.* (2012) observed a parabolic effect for rosmarinic alkyl esters in emulsions prepared with Tween-20 whereas, in our case, alkyl gallates in emulsions prepared with Tween-20 seem to follow better the behaviour predicted by the polar paradox. It seems to be related to the interactions of the emulsifier and a specific antioxidant than to the existence of a universal emulsifier for all antioxidants.

The relevance of the nature of the emulsifier in emulsions has been studied by Stöckmann *et al.* (2000). They reported that the antioxidant activity of a short homologous alkyl gallate series (from gallic acid to octyl gallate) in stripped corn oil-in-water emulsions showed great differences depending on the emulsifier used (lecithin, SDS and Brij 58). They hypothesized that specific molecular interactions between the antioxidants and the emulsifier were the cause of the differences found between emulsions. They proposed that these interactions could be between the antioxidant and the headgroup of the emulsifier (e.g. hydrogen bonds between the phenolic OH groups and the charge of the emulsifier) and also between the alkyl chains of the antioxidant and the lipid chain of the emulsifier, which would

affect the diffusion of the antioxidant in the emulsion. Several other authors (Aleman *et al.*, 2015; McClements *et al.*, 2000; Shahidi *et al.*, 2011; Sørensen *et al.*, 2008) have also suggested the relevance of the interactions between the emulsifiers and the antioxidants.

Additionally, an important aspect that could be related to the differences found between the antioxidant activity of the gallate derivatives in emulsions stabilized with lecithin, Tween-20 and SDS, is the significance of the antioxidant properties of emulsifiers for improving the oxidative stability of emulsions. Lecithin is a known antioxidant compound with good emulsification properties (Judde *et al.*, 2003). In contrast, Tween-20 and SDS are emulsifiers without any known antioxidant properties due to lack of functional groups responsible for antioxidant activity (Kerwin, 2008). Pan *et al.* (2013) have demonstrated a major stabilization of emulsions with lecithin associated to a lower rate of permeation of peroxyl radicals from the aqueous phase to the oil phase of emulsion compared with emulsions stabilized with Tween-20. The higher rate of permeation of peroxyl radicals in the Tween-20 emulsions, due to the minor antioxidant activity of this emulsifier provoked a destabilization of the emulsion in terms of oxidation. Therefore, in our work, probably antioxidant synergistic or additive effects between lecithin and the gallate antioxidants are occurring and contributing to the antioxidant effectiveness identified for each gallate derivative. Such synergistic and additive effects could be dependent of the molecular structure of the conjugated gallate antioxidant. Tween-20 and SDS are compounds with no known antioxidant properties, thus they cannot increase or decrease the antioxidant activity associated to the gallate derivatives.

In this work we designed glycosyl alkyl gallates to improve the surface active properties of the corresponding alkyl gallates expecting also to improve their antioxidant activity in emulsions (since they still possess a di-ortho phenolic unit in their structure). When we measured the surface tension in aqueous solutions for glycosyl alkyl gallates we found that from the butyl (**11**) to the dodecyl (**15**) derivatives these compounds behave as surfactants (Table S10) (Maldonado *et al.*, 2011). Moreover, the surfactant effectiveness (γ_{cmc}, surface tension at the CMC) for compounds **11**-**15** is lower than for the corresponding alkyl gallates **4**-**6** (**7** and **8** do not behave as surfactants) demonstrating that they are

better surfactants. However, glycosyl alkyl gallates displayed less antioxidant efficiency in oil-in-water emulsions than alkyl gallates. These results were somehow surprising since the structure of these compounds still maintain a di-ortho phenolic ring and better antioxidant activity could be expected. It is important to note that the ester functionality in these alkyl gallate derivatives partially deactivates the aromatic ring what can limit the hydrogen donating capacity of the phenolic OH groups and decreases the stability of a radical on the ring. In fact, other antioxidants with a di-ortho phenolic moiety and an electron-rich aromatic ring such as hydroxytyrosol display excellent antioxidant activity (Medina *et al.*, 2009).

When the percentage of oxidation inhibition was plotted against the alkyl chain length for each glucosyl alkyl gallate (Figure 2B), we observed a similar scenario to that found for the alkyl gallates, butyl gallate and medium-size alkyl gallates (C6-C10) were the best antioxidants in emulsions prepared with lecithin whereas in emulsions prepared with Tween-20 the glucosyl phenolipids tend to follow the polar paradox.

Finally, direct comparison of antioxidants containing the same hexadecyl alkyl chain and different polar headgroups, galloyl- **7**, glucosylgalloyl- **14**, glucuronosylgalloyl- **16** and glucuronate methyl ester galloyl- **17**, indicates again the relevance of maintaining the three OH groups in the aromatic ring and also points out the glycosyl unit is not relevant for activating the radical scavenging activity of the phenolic group or for the location in the interphase of the emulsion since we only observe minor differences among them. Moreover, the fact that glucuronosyl hexadecyl gallate **16** shows surfactant properties does not improve its antioxidant activity when compared to **14** and **17** which are not surfactants.

In conclusion, we have found that maintenance of the three phenolic hydroxyl groups in gallic acid is fundamental for the antioxidant efficiency of alkyl gallate derivatives since glycosylation of just one OH group results in a large decrease in antioxidant capacity. Improvement of the surfactant properties of the alkyl gallate by addition of a carbohydrate in their polar head does not translate in better antioxidant efficiency. The type of emulsifier seems to be playing an important role and probably

specific interactions between emulsifier and antioxidants together to the additive or synergistic effect occurring may rule their antioxidant activity in oil-in-water emulsions. Strong head-to-head and tail-to-tail interactions between the emulsifier and the phenolipid may place the antioxidant closer to the interphase and therefore could display better protecting efficiency in the emulsion. If these interactions are weaker then the antioxidant will tend to be more randomly located in the emulsion affecting its antioxidant activity. Finally, small differences in antioxidant efficiency were observed when glucosyl, glucuronosyl and glucuronosyl methyl ester hexadecyl gallates were compared.

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LITERATURE CITED

Aleman, M., Bou, R., Guardiola, F., Durand, E., Villeneuve, P. *et al.* (**2015**). Antioxidative effect of lipophilized caffeic acid in fish oil enriched mayonnaise and milk. *Food Chem, 167*, 236-244.

Bang, H. O., Dyerberg, J.Nielsen, A. B. (**1971**). Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet, 1*(7710), 1143-1145.

Chaiyasit, W., McClements, D. J.Decker, E. A. (**2005**). The relationship between the physicochemical properties of antioxidants and their ability to inhibit lipid oxidation in bulk oil and oil-in-water emulsions. *J Agric Food Chem, 53*(12), 4982-4988.

Chapman, R. A.Mackay, K. (**1949**). The estimation of peroxides in fats and oils by the ferric thiocyanate method. *J. Am. Oil Chem. Soc., 26*, 360-363.

Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S.Vane, J. R. (**1978**). Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet, 2*(8081), 117-119.

Evans, E. I. (**1935**). Antioxidant properties of vegetable lecithin. *Ind. Eng. Chem., 27*, 329-331.

Feigenbaum, J. (**1946**). Antioxidant effect of commercial lecithin in fortified margarine. *Nature, 157*, 770–771.

Fendler, J. H. (1982). *Membrane Mimetic Chemistry*. New York: John Wiley and Sons.

Frankel, E. N. (1998). *Lipid oxidation*. Dundee, Scotland: Oily Press.

Frankel, E. N., Huang, S.-W., Kanner, J.German, J. B. (**1994**). Interfacial Phenomena in the Evaluation of Antioxidants: Bulk Oils vs Emulsions. *J. Agric. Food Chem., 42*(5), 1054-1059.

Heins, A., McPhail, D., Sokolowski, T., Stöckmann, H.Schwarz, K. (**2007**). The Location of Phenolic Antioxidants and Radicals at Interfaces Determines Their Activity. *Lipids, 42*(6), 573-582.

Hsieh, R. J.Kinsella, J. E. (**1989**). Oxidation of polyunsaturated fatty acids: mechanisms, products, and inhibition with emphasis on fish. *Adv Food Nutr Res, 33*, 233-341.

Huang, S.-W., Frankel, E. N., Schwarz, K., Aeschbach, R.German, J. B. (**1996a**). Antioxidant Activity of Carnosic Acid and Methyl Carnosate in Bulk Oils and Oil-in-Water Emulsions. *J Agric Food Chem, 44*(10), 2951-2956.

Huang, S.-W., Hopia, A., Schwarz, K., Frankel, E. N.German, J. B. (**1996b**). Antioxidant Activity of alfa-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils vs Oil-in-Water Emulsions. *J Agric Food Chem, 44*(2), 444-452.

Iglesias, J., Lois, S.Medina, I. (**2007**). Development of a solid-phase microextraction method for determination of volatile oxidation compounds in fish oil emulsions. *J Chromatogr A, 1163*(1-2), 277-287.

Judde, A., Villeneuve, P., Rossignol-Castera, A.Le Guillou, A. (**2003**). Antioxidant effect of soy lecithins on vegetable oil stability and their synergism with tocopherols. *J. Am. Oil Chem. Soc., 80*, 1209–1215.

Kerwin, B. A. (**2008**). Polysorbates 20 and 80 used in the formulation of protein biotherapeutics: structure and degradation pathways. *J Pharm Sci, 97*(8), 2924-2935.

Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K.Taniguchi, H. (**2002**). Antioxidant properties of ferulic acid and its related compounds. *J Agric Food Chem, 50*(7), 2161-2168.

Laguerre, M., Bayrasy, C., Lecomte, J., Chabi, B., Decker, E. A. *et al.* (**2013**). How to boost antioxidants by lipophilization? *Biochimie*, 95(1), 20-26.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M.-C., Baréa, B. *et al.* (**2009**). Chain Length Affects Antioxidant Properties of Chlorogenate Esters in Emulsion: The Cutoff Theory Behind the Polar Paradox. *J Agric Food Chem, 57*(23), 11335-11342.

Laguerre, M., López Giraldo, L. J., Lecomte, J., Figueroa-Espinoza, M.-C., Baréa, B. *et al.* (**2010**). Relationship between Hydrophobicity and Antioxidant Ability of Phenolipids in Emulsion: A Parabolic Effect of the Chain Length of Rosmarinate Esters. *J Agric Food Chem, 58*(5), 2869-2876.

Losada-Barreiro, S., Sanchez-Paz, V.Bravo-Diaz, C. (**2013**). Effects of emulsifier hydrophile-lipophile balance and emulsifier concentration on the distributions of gallic acid, propyl gallate, and alpha-tocopherol in corn oil emulsions. *J Colloid Interface Sci, 389*(1), 1-9.

Lucas, R., Comelles, F., Alcantara, D., Maldonado, O. S., Curcuroze, M. *et al.* (**2010**). Surface-active properties of lipophilic antioxidants tyrosol and hydroxytyrosol fatty acid esters: a potential explanation for the nonlinear hypothesis of the antioxidant activity in oil-in-water emulsions. *J Agric Food Chem, 58*(13), 8021-8026.

Maldonado, O. S., Lucas, R., Comelles, F., Gonzalez, M. J., Parra, J. L. *et al.* (**2011**). Synthesis and characterization of phenolic antioxidants with surfactant properties: glucosyl- and glucuronosyl alkyl gallates. *Tetrahedron, 67*, 7268-7279.

McClements, D. J.Decker, E. A. (**2000**). Lipid Oxidation in Oil-in-Water Emulsions: Impact of Molecular Environment on Chemical Reactions in Heterogeneous Food Systems. *J Food Sci, 65*(8), 1270-1282.

Medina, I., Lois, S., Alcántara, D., Lucas, R.Morales, J. C. (**2009**). Effect of Lipophilization of Hydroxytyrosol on Its Antioxidant Activity in Fish Oils and Fish Oil-in-Water Emulsions. *J Agric Food Chem, 57*, 9773-9779.

Pan, Y., Tikekar, R. V.Nitin, N. (**2013**). Effect of antioxidant properties of lecithin emulsifier on oxidative stability of encapsulated bioactive compounds. *Int J Pharm, 450*(1-2), 129-137.

Panya, A., Laguerre, M., Bayrasy, C., Lecomte, J., Villeneuve, P. *et al.* (**2012**). An investigation of the versatile antioxidant mechanisms of action of rosmarinate alkyl esters in oil-in-water emulsions. *J Agric Food Chem, 60*(10), 2692-2700.

Porter, W. L. (1980). Recent trends in food applications of antioxidants. In P. Press (Ed.), *Autoxidation in food and biological systems*, (pp. 295-365). New York: Plenum Press.

Porter, W. L., Black, E. D.Drolet, A. M. (**1989**). Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *J Agric Food Chem, 37*(3), 615-624.

Shahidi, F.Zhong, Y. (**2011**). Revisiting the polar paradox theory: a critical overview. *J Agric Food Chem, 59*(8), 3499-3504.

Sokal, R.Rohlf, F. (1994). *Biometry*. San Francisco, CA: W. H. Freeman.

Sørensen, A. D., Haahr, A. M., Becker, E. M., Skibsted, L. H., Bergenstahl, B. *et al.* (**2008**). Interactions between iron, phenolic compounds, emulsifiers, and pH in omega-3-enriched oil-in-water emulsions. *J Agric Food Chem, 56*(5), 1740-1750.

Sørensen, A. D. M., de Diego, S., Petersen, L. K., Nielsen, N. S., Yang, Z. *et al.* (**2010**). Effect of lipophilization of dihydrocaffeic acid on its antioxidative properties in fish oil enriched emulsion. *Proceedings of the 8th Euro Fed Lipid Congress*, 21-24.

Sørensen, A. D. M., Nielsen, N. S., Decker, E. A., Let, M. B., Xu, X. *et al.* (**2011**). The Efficacy of Compounds with Different Polarities as Antioxidants in Emulsions with Omega-3 Lipids. *J. Am. Oil Chem. Soc., 88*, 489-502.

Stöckmann, H., Schwarz, K.Huynh-Ba, T. (**2000**). The influence of various emulsifiers on the partitioning and antioxidant activity of hydroxybenzoic acids and their derivatives in oil-in-water emulsions. *J. Am. Oil Chem. Soc., 77*(5), 535-542.

Torres de Pinedo, A., Peñalver, P.Morales, J. C. (**2007a**). Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship. *Food Chem, 103*, 55-61.

Torres de Pinedo, A., Peñalver, P., Pérez-Victoria, I., Rondón, D.Morales, J. C. (**2007b**). Synthesis of new phenolic fatty acid esters and their evaluation as lipophilic antioxidants in an oil matrix. *Food Chem, 105*, 657-665.

Tziomalos, K., Athyros, V. G.Mikhailidis, D. P. (**2007**). Fish oils and vascular disease prevention: an update. *Curr Med Chem, 14*(24), 2622-2628.

Yuji, H., Weiss, J., Villeneuve, P., Lopez Giraldo, L. J., Figueroa-Espinoza, M. C. *et al.* (**2007**). Ability of surface-active antioxidants to inhibit lipid oxidation in oil-in-water emulsion. *J Agric Food Chem, 55*(26), 11052-11056.

Zhong, Y.Shahidi, F. (**2012**). Antioxidant behavior in bulk oil: limitations of polar paradox theory. *J Agric Food Chem, 60*(1), 4-6.

FIGURE LEGENDS

Figure 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**.

Figure 2. A) Percentage of inhibition of gallic acid **1** and alkyl gallates **2-8** vs their alkyl chain length. Symbol ■ represents % inhibition at day 7 at 45 °C using lecithin as emulsifier. Symbol **x** represents % inhibition at day 8 at 30 °C using Tween-20 as emulsifier. B) Percentage of inhibition of gallic acid **1** and glucosyl alkyl gallates **9-15** vs their alkyl chain length. Symbol ■ represents % inhibition at day 7 at 30 °C using lecithin as emulsifier. Symbol **x** represents % inhibition at day 4 at 30 °C using Tween-20 as emulsifier.

Table 1. Inhibition by gallic acid **1** and alkyl gallates **2-8** on the formation of conjugated diene and triene hydroperoxides in fish oil-in-water emulsions during oxidation (Tween-20 was used as emulsifier at 30 ºC, lecithin was used as emulsifier at 45ºC). Antioxidants were tested at the same concentration: 0.1 mmol/kg (mean±sd) [1,2].

Phenolic antioxidants	Tween-20		Lecithin			
	Conj. Dienes (day 4)	Conj. Trienes (day 4)	Conjugates Dienes (day 7)	Conjugates Dienes (day 10)	Conjugated Trienes (day 7)	Conjugated Trienes (day 10)
Control	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.1^{a}	0.0 ± 0.0^{a}
Gallic acid **1**	-26.9 ± 2.8^{a}	-10.3 ± 0.4^{a}	22.0 ± 2.8^{b}	-95.7 ± 21.2^{a}	21.1 ± 2.5^{b}	-62.7 ± 4.2^{a}
Butyl gallate **2**	54.4 ± 0.4^{b}	74.2 ± 3.5^{b}	73.6 ± 3.9^{d}	-25.3 ± 7.0^{a}	78.6 ± 1.5^{d}	11.5 ± 1.5^{b}
Hexyl gallate **3**	88.5 ± 0.4^{d}	91.7 ± 0.9^{c}	84.8 ± 3.2^{e}	65.2 ± 12.9^{d}	87.2 ± 5.3^{e}	81.1 ± 8.3^{d}
Octyl gallate **4**	89.4 ± 1.1^{d}	93.2 ± 2.8^{d}	80.7 ± 8.5^{de}	37.0 ± 2.6^{c}	80.0 ± 10.9^{e}	67.2 ± 11.3^{cd}
Decyl gallate **5**	85.8 ± 0.5^{c}	87.6 ± 1.3^{c}	81.9 ± 2.8^{e}	19.6 ± 3.8^{b}	81.7± 5.0^{e}	58.6 ± 1.6^{cd}
Dodecyl gallate **6**	92.3 ± 1.4^{e}	96.3 ± 0.9^{e}	84.2 ± 3.6^{e}	60.9 ± 1.8^{d}	84.8 ± 10.6^{e}	78.0 ± 1.4^{cd}
Hexadecyl gallate **7**	91.0 ± 1.0^{e}	95.4 ± 2.1^{e}	74.4 ± 7.2^{de}	19.9 ± 2.6^{b}	74.3 ± 6.8^{de}	55.0 ± 9.3^{c}
Octadecyl gallate **8**	92.2 ± 1.5^{e}	96.4 ± 0.6^{e}	59.5 ± 4.2^{c}	-17.1 ± 8.0^{a}	60.8 ± 4.2^{c}	10.0 ± 3.1^{b}

[1] % Inhibition = [(C - S)/C] X 100 where C = increment in the oxidation product formed in control and S = increment in the oxidation product formed in sample (Frankel, 1998). [2]Values in each column with the same superscript letter were not significantly different ($p < 0.01$).

Table 2. Inhibition by gallic acid **1** and glucosyl alkyl gallates **9**-**15** on the formation of conjugated diene and triene hydroperoxides in fish oil-in-water emulsions during oxidation at 30ºC using lecithin (data on day 8) or Tween-20 (data on day 4) as emulsifiers. Antioxidants were tested at the same concentration: 0.1 mmol/kg (mean±sd) [1,2].

Phenolic antioxidants	Tween-20		Lecithin	
	Conj. Dienes	Conj. Trienes	Conj. Dienes	Conj. Trienes
Control	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	0.0 ± 0.0^{a}
Gallic acid **1**	-26.9 ± 2.8^{a}	-10.3 ± 0.4^{a}	32.1 ± 0.9^{b}	26.9 ± 1.5^{c}
Glc-butyl gallate **9**	5.0 ± 0.2^{b}	6.0 ± 1.2^{b}	49.6 ± 1.0^{d}	47.4 ± 2.3^{e}
Glc-hexyl gallate **10**	28.3 ± 0.5^{e}	33.6 ± 0.5^{d}	31.5 ± 0.4^{b}	26.6 ± 0.8^{c}
Glc-octyl gallate **11**	41.6 ± 0.8^{f}	43.1 ± 0.5^{e}	38.9 ± 1.2^{c}	39.8 ± 1.8^{d}
Glc-decyl gallate **12**	12.4 ± 1.1^{c}	8.6 ± 0.6^{c}	30.7 ± 0.9^{b}	27.8 ± 1.1^{c}
Glc-dodecyl gallate **13**	32.6 ± 3.2^{e}	50.8 ± 5.5^{f}	-2.3 ± 0.8^{a}	-2.5 ± 1.5^{a}
Glc-hexadecyl gallate **14**	22.7 ± 0.4^{d}	66.0 ± 0.7^{g}	-6.1 ± 0.9^{a}	3.5 ± 0.2^{b}
Glc-octadecyl gallate **15**	56.3 ± 5.3^{g}	57.6 ± 0.9^{f}	-9.1 ± 0.9^{a}	-5.6 ± 0.1^{a}

[1] % Inhibition = [(C - S)/C] X where C = increment in the oxidation product formed in control and S = increment in the oxidation product formed in sample (Frankel, 1998). [2]Values in each column with the same superscript letter were not significantly different ($p < 0.01$).

532 **Table 3.** Inhibition by octyl gallate **4**, hexadecyl gallate **7**, glucosyl hexadecyl gallate **14**, glucuronosyl
533 alkyl gallates **16** and glucuronosyl methyl ester hexadecyl gallate **17** on the formation of conjugated
534 diene and triene hydroperoxides in fish oil-in-water emulsions during oxidation at 50ºC using lecithin as
535 emulsifier (data on day 4) and during oxidation at 30ºC using Tween-20 as emulsifier (data on day 4).
536 Antioxidants were tested at the same concentration: 0.1 mmol/kg (mean±sd) [1,2].

537

Phenolic antioxidants	Tween-20		Lecithin	
	Conj. Dienes	Conj. Trienes	Conj. Dienes	Conj. Trienes
Control	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}	0.0 ± 0.1^{a}
Octyl gallate **4**	89.4 ± 1.1^{b}	93.2 ± 2.8^{b}	91.10 ± 0.34^{b}	89.89 ± 1.10^{b}
Hexadecyl gallate **7**	91.0 ± 1.0^{b}	95.4 ± 2.1^{b}	67.53 ± 3.48^{c}	68.95 ± 4.01^{c}
Glc-hexadecyl gallate **14**	22.7 ± 0.4^{d}	49.3 ± 0.7^{c}	35.04 ± 6.22^{d}	43.56 ± 4.51^{d}
GlcA-hexadecyl gallate **16**	37.5 ± 0.2^{b}	50.4 ± 0.9^{c}	33.28 ± 2.42^{d}	41.76 ± 0.13^{d}
MeGlcA-hexadecyl gallate **17**	25.2 ± 0.9^{c}	38.4 ± 1.3^{d}	14.71 ± 2.47^{e}	14.71 ± 6.65^{e}

538 [1] % Inhibition = [(C - S)/C] X 100 where C = increment in the oxidation product formed in control and S = increment
539 in the oxidation product formed in sample (Frankel, 1998). [2]Values in each column with the same superscript letter were
540 not significantly different ($p < 0.01$).

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542

543 TOC **Graphic**

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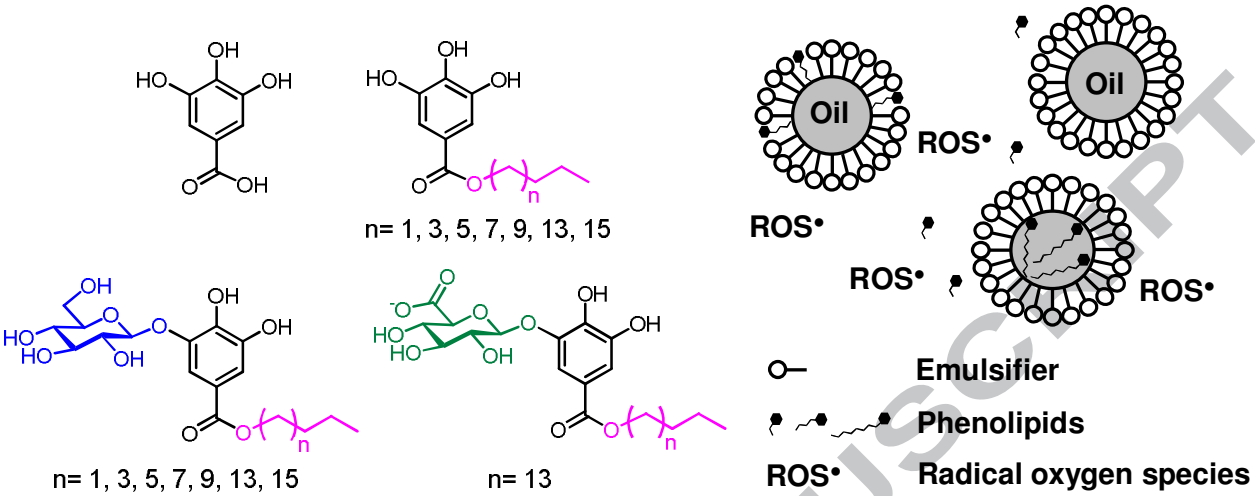

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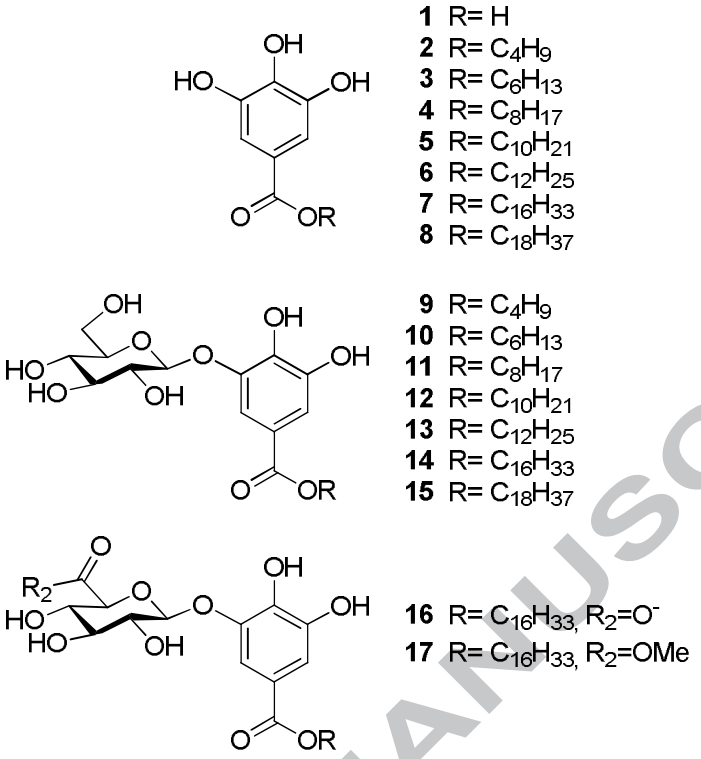
546

555 **Figure 1.**



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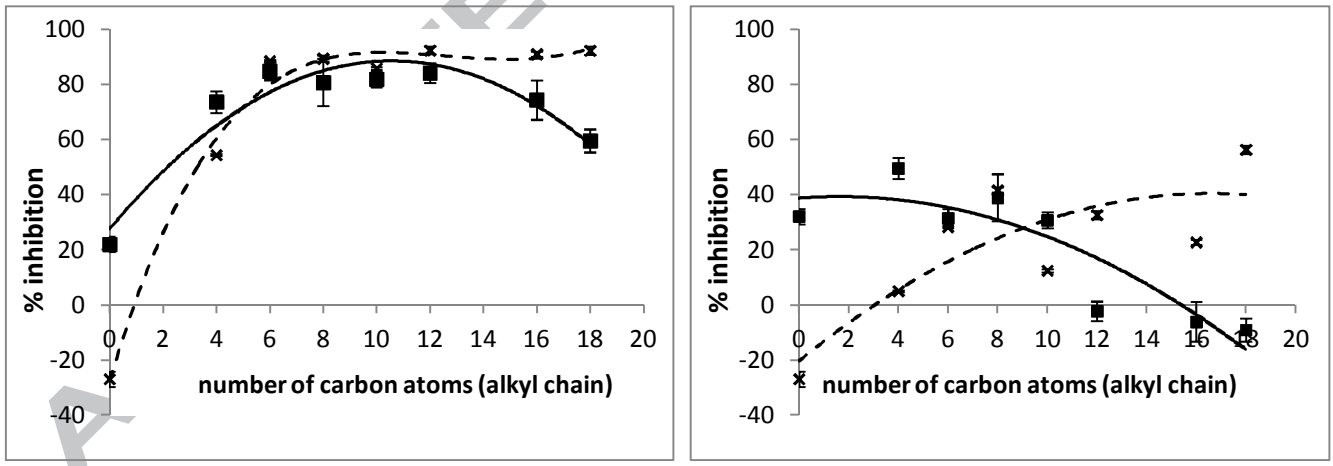
557 **Figure 2.**

558 A) B)



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