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ORIGINAL PAPER

Enzymatic Synthesis of Tyrosol-Based Phenolipids: Characterization and Effect of Alkyl Chain Unsaturation on the Antioxidant Activities in Bulk Oil and Oil-in-Water Emulsion

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Abstract Oxidative stability of lipids is one of the most important parameters affecting their quality. Lipase-catalyzed lipophilic tyrosyl esters with an equivalent carbon alkyl chain but different degrees of unsaturation (C18:0 to C18:4n3) were prepared, characterized, and used as antioxidants. Free fatty acids and fatty acid ethyl esters (substrate molar ratio tyrosol: acyl donor, 1:10) were used as acyl donors and immobilized lipase from *Candida antarctica* was the biocatalyst (10 %). The phenolipids were isolated and characterized using ESI–MS, ^{1}H-NMR, and ^{13}C-NMR. Peroxide value (PV) and *para*-anisidine value (*p*-AV) were measured to evaluate their antioxidant activities in bulk oil structured lipid (SL) and in an oil-in-water emulsion (SL-based infant formula). No significant difference was found in yield and reaction time between the two types of acyl donors. However, as the unsaturation of the fatty acids increased the reaction time also increased. In SL, tyrosyl esters exhibited lower antioxidant activity than tyrosol whereas the addition of an alkyl chain enhanced the antioxidant efficiency of tyrosol in infant formula. Tyrosyl oleate was the most efficient antioxidant in the emulsion system followed by tyrosyl stearate and tyrosyl linoleate. These results suggest that the synthesized phenolipids may be used as potential antioxidants in lipid-based products.

Keywords Phenolipids · Tyrosol · Antioxidant · Structured lipids · Infant formula

Introduction

Food lipids are a significant functional and healthful constituent of various food products. They affect the chemical and physical properties of food such as texture and flavor and also have a major effect on human health. However, most oils and fats are susceptible to oxidation which leads to the formation of off-flavors and potentially harmful products [1]. Oxidation in food affects its flavor, nutritional quality, and functional attributes. Antioxidants are widely used in the manufacture, packaging, and storage of lipid-containing foods. Much interest has developed during the last few decades in naturally occurring antioxidants because of the adverse attention received by synthetic antioxidants, and also the worldwide trend to avoid or minimize the use of artificial food additives [2].

Phenolic compounds are secondary metabolites produced by plants and reported to exhibit several health effects [3]. However, due to their highly polar nature their use in lipid-based food products is limited. "Phenolipids" are lipophilized phenolic derivatives produced with an aim to improve the hydrophobicity of the phenolic moiety and its efficiency in lipid-based systems. These are amphiphilic in nature as they can be used in both lipid and oil-in-water emulsion systems. A number of structural factors can affect the antioxidative activity of the phenolipids such as the number of phenolic hydroxyl group, presence of primary hydroxyl group, and nature of the alkyl chain [4]. Phenolipids can be prepared by either esterification of phenolic acids (–COOH) with fatty alcohols [5] or grafting a fatty acid to the phenolic alcohol (–OH) [1]. The reactions

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employed can be esterification with free acids or transesterification with methyl or ethyl esters.

Tyrosol and hydroxytyrosol are two characteristic phenolic compounds present in olive oil. Their free forms as well as their secoroid derivatives constitutes around 30 %, and other conjugated forms such as oleuropein and ligstroside aglycones represent almost half of the total phenolic content of a virgin olive oil [6]. Olive oil phenolics have beneficial biological effects [7] and also contribute to the high stability of olive and other oils [8]. Several studies have been done on the synthesis of phenolipids from hydroxytyrosol and tyrosol [9–16]. These studies have focused on the effect of increasing chain length/lipophilicity on the antioxidant or antimicrobial [13] activities of the phenolipids. In this paper, we used the same chain length but increasing degree of unsaturation (C18:0 to C18:4). Trujillo *et al*. [9] reported that esterification does not affect the antioxidant activity of hydroxytyrosol in lipid matrices however antioxidant capacities of the lipophilic esters were enhanced in a biological model. A number of studies have reported the existence of a critical chain length or cut-off effect of the synthesized lipophilic phenolic esters to achieve maximum antioxidant capacity [12, 13, 17–19]. The effect of chain length on the antioxidant ability of rosmarinate esters [17] and chlorogenate esters [18] on tung oil based emulsion using conjugated autoxidizable triene assays, showed the nonlinear behavior and cut-off theory of the esters. The polar paradox hypothesis explains that hydrophilic antioxidants are more efficient in bulk oils whereas lipophilic antioxidants work better in emulsions and micelles. Recent studies have shown that not all antioxidants follow this hypothesis in oil and emulsion systems [20]. Most studies have focused on the effect of varying chain lengths whereas a few have shown the difference of behavior between saturated and unsaturated long chain ester derivatives. In this paper we aimed at synthesizing lipophilic tyrosyl esters with increasing degree of unsaturation but with the same chain length. To this end, we evaluated both free fatty acids and fatty acid ethyl esters as acyl donors. The antioxidant efficiency of the synthesized esters were tested in bulk oil structured lipid (SL) and in an oil-in-water emulsion (SL-based infant formula) using peroxide value (PV) and *para*-anisidine value (*p*-AV). In a previous study by our group [21], it was observed that during SL synthesis and purification the phenolics of the substrate oil were lost in free and/or esterified form. Therefore, adding them back as phenolipids can help in SL stability. With the growing trend of clean label and natural ingredients, use of antioxidants derived from indigenous components of olive oil in olive oil-based SL can be a potential use of such phenolipids.

Materials and Methods

Materials

Tyrosol was obtained from Oakwood Products Inc. (West Columbia, SC, USA). Stearic, oleic, linoleic, α-linolenic acids, and their ethyl esters (≥98 %) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Stearidonic acid and its ethyl ester (>98 %) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The immobilized enzyme Novozym® 435 (*Candida antarctica* lipase, non-specific, specific activity 10,000 PLU/g: PLU = Propyl Laurate Unit) was purchased from Novozymes North America Inc. (Franklinton, NC, USA). Tetrahydrofuran (THF, ≥99 %, anhydrous, inhibitor-free) was purchased from Sigma-Aldrich Chemical Co. Other solvents and chemicals were from Fisher Scientific (Norcross, GA, USA) and Sigma-Aldrich Chemical Co.

Preparation of Phenolipids

Phenolipid synthesis was performed in screw-cap test tubes. First, 0.1 g of tyrosol was reacted with various acyl donors (C18:0–C18:4, free and ethyl esters) in a 1:10 substrate molar ratio (tyrosol: acyl donor). Then, 5 mL THF was added and the substrates were thoroughly mixed. Novozym 435 lipase was added at 10 % total substrate weight. Molecular sieves (3 Å, 4–8 mesh) were also added (5 % by weight) to the test tubes to eliminate any moisture formed during the reactions. The tubes were flushed with nitrogen and the reactions were carried out at 50 °C for 24 h under constant stirring. Samples were analyzed at 0, 1, 4, 12, and 24 h for reaction completion. Reactions were monitored by analyzing tyrosol using a high-performance liquid chromatograph (HPLC; Agilent Technologies 1260 Infinity, Santa Clara, CA, USA) equipped with a Sedex 85 evaporative light scattering detector (ELSD) at 70 °C and 3.8 bar and UV–DAD at 240 and 260 nm. A Beckman Ultrasphere® C18 column, 5 μm, 4.6 × 250 mm was used with the temperature set at 30 °C. The injection volume was 20 μL. The mobile phase at a flow rate of 1 mL/min consisted of methanol/acetonitrile/water (95:3:2, by volume) [22]. The reaction was stopped and the mix was filtered through anhydrous sodium sulfate column to remove the enzymes. Pure tyrosyl esters were obtained using silica solid phase extraction (SPE) by eluting with hexane/*tert*-butyl methyl ether (8:1–10:1) [10]. The samples were dried under nitrogen and reconstituted in 2 mL methanol and stored in −20 °C until further analyses.

Characterization of Phenolipids

For electrospray ionization mass spectrometry (ESI–MS) analysis, the sample (10 µL) was dissolved in 990 µL of methanol–isopropyl alcohol–propyl alcohol 16:3:3 mixture (infusion buffer). ESI–MS analysis was performed by direct infusion (DI) on an LCQ mass-spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA) equipped with electrospray ion source. The samples were infused directly into the instrument at a constant flow rate of 5 µL/min. The capillary temperature was set at 275 °C and MS analysis was performed in the negative ion mode in *m/z* range of 100–1500. A full FT-MS spectrum was collected at 30,000 resolution, and for MS/MS analysis, the collision energy was set at 35 eV. For NMR sample preparation, methanol was removed by evaporation under N_2-stream and the samples were dissolved in $CDCl_3$ (deutero-chloroform). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 600 MHz spectrometer (Bruker BioSpin Corporation, Billerica, MA, USA) equipped with a 5-mm probe at 25 °C, in $CDCl_3$. Chemical shifts were referenced to residual methanol peak (δ_H/δ_C 3.35/48.8 ppm).

Oxidation Study

The peroxide value (PV) and *para*-anisidine value (*p*-AV) were measured to evaluate their antioxidant activities in bulk oil (SL) and in an oil-in-water emulsion, o/w (SL-based infant formula, IF). The SL used as the lipid phase in the infant formula was prepared using a one-pot synthesis design using tripalmitin, extra virgin olive oil, and free fatty acids of arachidonic and docosahexaenoic single cell oils in the ratio of 0.5:1:0.5 [21]. Both enzymes Novozym 435 and Lipozyme TL IM lipases were added simultaneously and the reaction was carried out for 24 h at 60 °C [21]. After the reaction, the extra FFA were removed and the purified SL was used for oxidation study and infant formula preparation. Infant formula was prepared as described by Zou and Akoh [23]. Briefly, high heat nonfat dry milk, [alpha]-lactalbumin enriched whey protein concentrate, lactose, and carrageenan were fully dissolved in water and lecithin was dispersed in SL. The oil phase was added to the aqueous phase while stirring at 60 °C and micronutrient premix was added. Polytron high-speed batch disperser (Kinematica, Inc., Bohemia, NY, USA) Polytron high-speed batch disperser (Kinematica, Inc., Bohemia, NY, USA) at setting 5 for 2 min and EmulsiFlex-C5 high-pressure homogenizer (Avestin, Inc., Ottawa, Canada) at 5000–7000 psi (34.47–48.26 MPa) were used to prepare the emulsion. After pH adjustment, the resulting emulsion was sterilized in an autoclave at 121 °C for 6 min 100 ppm of the tyrosyl esters were added to the samples and stored for 21 days at 45 °C in the dark in a shaking water bath at 200 rpm. Different samples were prepared for 7, 14, and 21 days analyses. Control samples (containing no added antioxidants) were also prepared. The PV and *p*-AV were determined according to AOCS Official Method Cd 8b-90 and AOCS Official Method Cd18-90, respectively [24]. Total oxidation (TOTOX) value was calculated as (2 × PV) + *p*-AV [25].

Statistical Analysis

All reactions and analyses were performed in duplicate and averages reported. Statistical analysis was performed with the SAS software package (SAS Institute, Cary, NC, USA). Duncan's multiple-range test was performed to determine the significant difference ($P \leq 0.05$) between the tyrosyl esters.

Results and Discussion

Preparation and Characterization of Phenolipids

The lipophilic esters of tyrosol were prepared with two types of acyl donors, namely, free forms and ethyl esters of stearic, oleic, linoleic, linolenic, and stearidonic acids. The reactions were monitored for tyrosol disappearance at regular intervals. However, complete disappearance of tyrosol was not obtained during the monitored reaction time. Therefore, the reactions were stopped when a stable level of tyrosol was achieved. There was no significant difference found between the two types of acylating agents in terms of yield and reaction time. Previous studies have reported that water formed during esterification reaction can lower the yield as it facilitates hydrolysis of formed ester [13]. However, in this study molecular sieves were added to absorb any water formed during esterification reactions. Reaction time increased from stearic acid (6 h) to stearidonic acid (22 h). For oleic, linoleic, and linolenic acids the reactions were carried out for 10, 12, and 17 h, respectively. The yields of the tyrosyl esters (measured gravimetrically after SPE) were 71.6 ± 2.2a, 69.2 ± 1.8a, 64.8 ± 1.3b, 60.4 ± 2.6c, and 54.7 ± 2.1d % for stearate, oleate, linoleate, linolenate, and stearidonate esters, respectively. Saturated fats have a straight chain-like structure which allows them to stack tightly and have more intermolecular interactions. Unsaturated fatty acids are not linear and the double bonded carbons results in a bent chain structure which may cause steric hindrance and decrease close intermolecular interactions with tyrosol. Similar results were reported by Zhong and Shahidi while synthesizing epigallocatechin gallate esters with stearic (yield 56.9 %), eicosapentaenoic (yield 42.7 %), and docosahexaenoic (yield 30.7 %) acids [26]. In another study, higher yield and shorter reaction time was achieved using ethyl

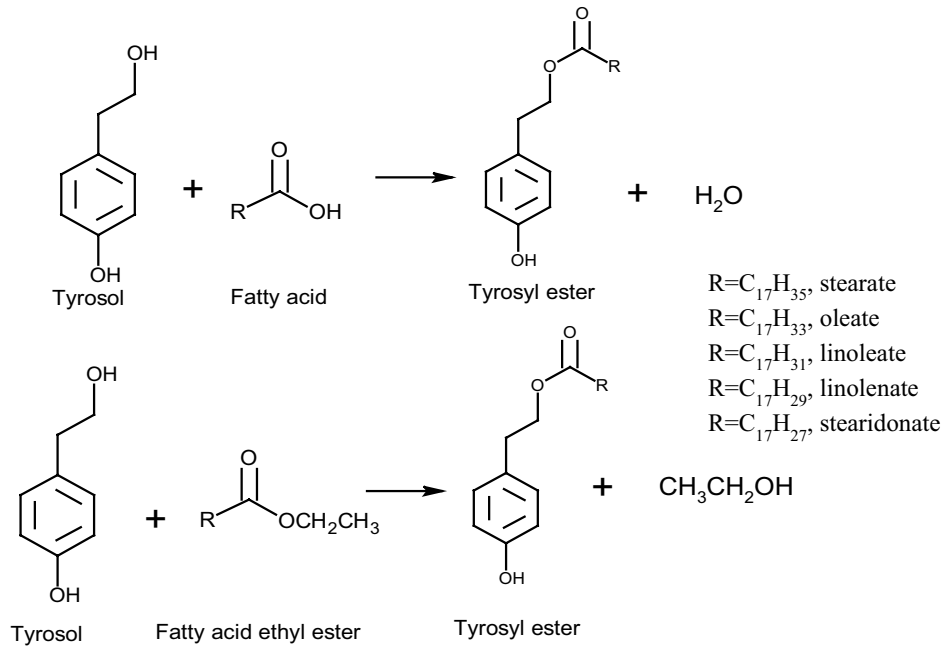
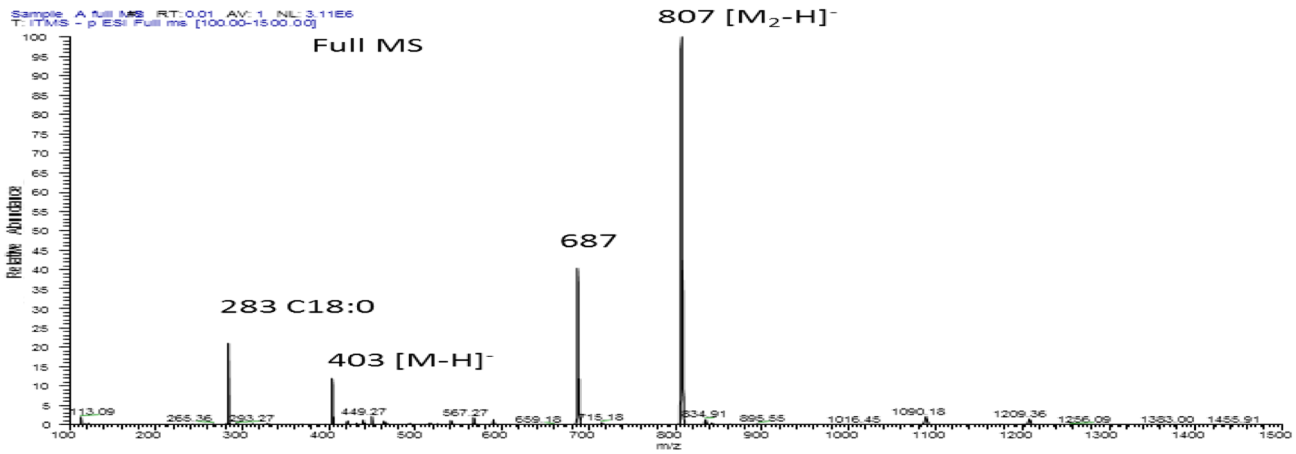


Fig. 1 Reaction scheme for synthesizing lipophilic tyrosol esters. Reactions were carried out in tetrahydrofuran with Novozym 435 lipase as biocatalyst at 50 °C for 24 h



Sample A

Fig. 2 Representative ESI–MS spectrum of phenolipid product A (tyrosyl stearate). For additional spectra, refer to the Supplementary Material

esters than free fatty acids. However, as the length of the fatty acids increased from stearic to docosahexaenoic acid, yield decreased significantly [27].

The reaction schemes are shown in Fig. 1. Tyrosol [4-(2-hydroxyethyl)phenol, $C_8H_{10}O_2$ MW 138.16 g/mol] reacts with different fatty acids (C18:0–C18:4n3) to form tyrosyl esters, namely tyrosyl stearate ($C_{26}H_{44}O_3$ MW 404.62 g/mol), tyrosyl oleate ($C_{26}H_{42}O_3$ MW 402.61 g/mol), tyrosyl linoleate ($C_{26}H_{40}O_3$ MW 400.59 g/mol), tyrosyl linolenate ($C_{26}H_{38}O_3$ MW 398.58 g/mol), and tyrosyl stearidonate ($C_{26}H_{36}O_3$ MW 396.56 g/mol). The prepared esters (samples A–E) were identified as specific tyrosol fatty acid esters by negative ion ESI–MS. The ESI–MS allows acquisition of mass spectra directly from aqueous samples with minimized fragmentation leaving mostly unaltered species. A representative ESI–MS spectrum is shown in Fig. 2. Full ESI–MS spectra can be found in the Supplementary Material. For sample A, the most dominant peak in the ESI–MS spectrum was the dimer $[M_2-H]^-$ located at 807 *m/z*. Phenolipid molecular ion $[M-H]^-$ and fatty acid molecular ion $[M-H]^-$ were identified at 403 and 283 *m/z*, respectively, confirming the sample to be tyrosyl stearate. In sample B, tyrosyl ester molecular ion $[M-H]^-$ was identified at 401 *m/z* and fatty acid molecular ion $[M-H]^-$ at 281 *m/z*, identifying the sample as tyrosyl oleate. Similarly, the analyzed and calculated *m/z* for tyrosyl ester molecular ions $[M-H]^-$ for samples C, D, and E were in accordance for tyrosyl linoleate (*anal.* 399,

Table 1 ^{1}H- and ^{13}C-NMR chemical shifts (δ, ppm) assignments for lipophilic tyrosol esters

Sample	b (CH_2)		c (CH_2)		d (CH_2)		e (Allylic)		f (Olefinic)		g (Bis allylic)		h (t-CH_3)		Compound
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	
A	2.30	34.1	1.61	25.0	1.28	29.4	–	–	–	–	–	–	0.89	13.7	Tyrosyl stearate
B	2.31	34.2	1.62	24.7	1.30	29.1	2.02	26.8	5.32	129.5	–	–	0.89	13.7	Tyrosyl oleate
C	2.29	34.2	1.62	24.7	1.33	29.1	2.05	26.8	5.34	129.6	2.78	25.2	0.89	13.5	Tyrosyl linoleate
D	2.30	34.4	1.62	24.9	1.33	28.9	2.06	27.0	5.35	127.6	2.82	25.3	0.97	13.7	Tyrosyl linolenate
E	2.32	33.5	1.63	24.0	1.37	28.4	2.08	26.6	5.26	128.5	2.76	25.3	0.98	13.1	Tyrosyl stearidonate

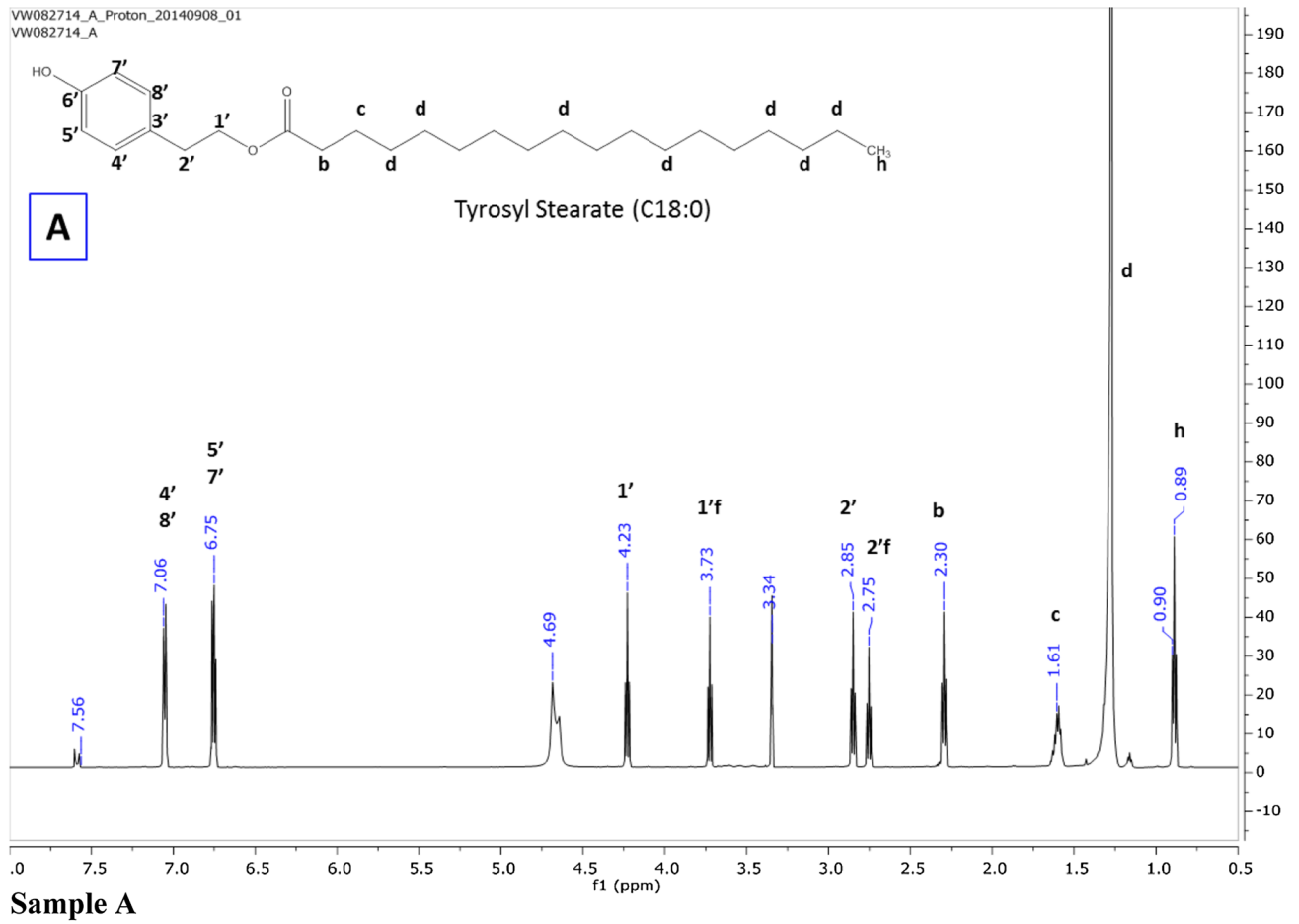


Fig. 3 Representative proton NMR spectrum of phenolipid product A (tyrosyl stearate). For additional spectra, refer to Supplementary Material

calc. 399.59) tyrosyl linolenate (*anal*. 397, *calc.* 397.58), and tyrosyl stearidonate (*anal.* 395, *calc*. 395.56). The random peaks detected in full MS spectra can be $[M-Ty]^-$ and fatty acid dimers.

^{1}H-NMR and ^{13}C-NMR analyses were used to confirm the structures from the results obtained from negative ion ESI–MS method. The results of chemical shift (δ, ppm) assignments for the tyrosyl esters are given in Table 1 and a representative proton NMR spectrum is shown in Fig. 3 (complete spectra can be found in Supplementary Material). For proton labels and assignments the structure for each sample is inserted on respective sample's proton spectrum. The numbers on the peaks represent chemical shift values. The proton NMR chemical shift is affected by proximity to electronegative atoms and unsaturated groups and the carbon NMR chemical shift is influenced by electronegative and steric effects. For ^{1}H-NMR, the proton chemical shift assignments (δ, ppm) for free tyrosol were 3.73 (1′), 2.73 (2′), 7.04 (4′, 8′), and 6.73 (5′, 7′) and for tyrosyl residue the chemical shifts were 4.21 (1′), 2.83 (2′),

7.04 (4′, 8′), and 6.73 (5′, 7′). A downward chemical shift was observed for 1′ (Δδ 0.48) and 2′ (Δδ 0.10) protons. In case of ^{13}C-NMR, the proton chemical shift assignments (δ, ppm) for free tyrosol were 63.5 (1′), 37.6 (2′), 129.4 (3′), 129.9 (4′, 8′), 114.7 (5′, 7′), and 155.3 (6′) and for tyrosyl residue the chemical shifts were 64.9 (1′), 34.3 (2′), 129.4 (3′), 129.9 (4′, 8′), 114.7 (5′, 7′), and 155.3 (6′). A larger downward chemical shift was observed for 1′ (Δ*δ* 1.4) and an upward shift was observed for 2′ (Δ*δ* 3.3) protons. The difference in shifts is because acylation on a hydroxyl group results in a downward chemical shift at the *O*-acyl carbon (1′ in tyrosyl residue) and an upward chemical shift at the adjacent carbon (2′ in tyrosyl residue) [28]. Similar trends in chemical shifts for ^{1}H-NMR and ^{13}C-NMR were reported by Zhong and Shahidi [26]. No chemical shift was detected in the aromatic ring protons. No allylic (e), bis-allylic (g) or olefinic protons (f) were detected in Samples A, confirming that the sample is tyrosyl stearate. No bis-allylic protons (g) were detected in Samples B but the presence of olefinic proton (f) signals indicated that sample B is tyrosyl oleate. Increasing intensity of bis-allylic (g), and decreasing intensity of methylene (d, CH2) proton signals in the proton spectra of C, D, and E, indicated progressing amount of double bonds in Samples C through E (C-tyrosyl linoleate, D-tyrosyl linolenate, E-tyrosyl stearidonate). These results are similar to those reported by Mateos *et al.* [10]. However, free tyrosol was observed in samples A and E but not in samples B, C, and D. Although the reactions were monitored for tyrosol disappearance but complete disappearance was not achieved and the reactions were stopped when a stable level of tyrosol was observed. Therefore, the samples may contain some tyrosol especially sample E because stearidonic acid due to steric hindrance had the longest reaction time and the lowest yield.

Oxidation Stability

The antioxidant effectiveness/efficiency of the synthesized tyrosyl esters were tested in bulk oil (SL) and an o/w emulsion (SL-based infant formula, IF). The major fatty acids in the SL were oleic (38.08 mol%), palmitic (35.23 mol%), arachidonic (6.23 mol%), and docosahexaenoic acids

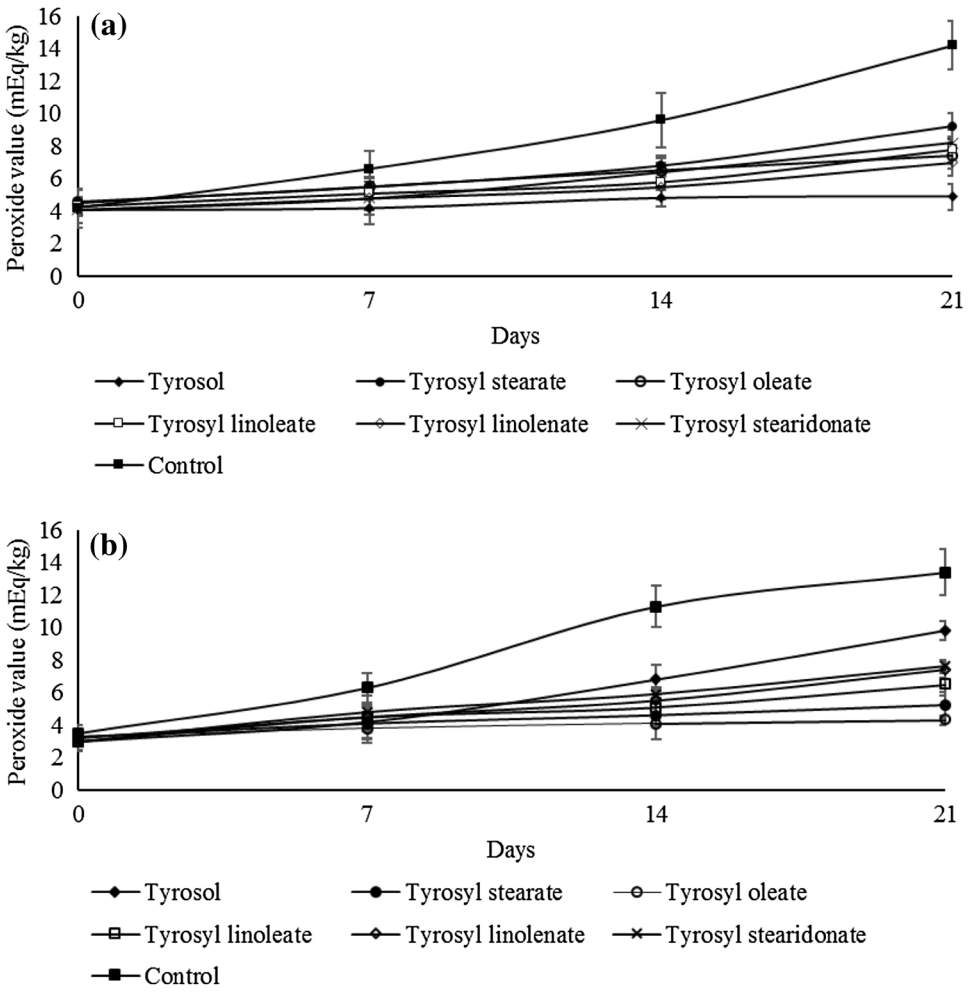


Fig. 4 Effect of lipophilic tyrosol esters on the oxidative stability of **a** bulk oil as structured lipid (SL) and **b** SL-based infant formula o/w emulsion measured by peroxide value over time at 45 °C Data points represent the average of duplicate samples

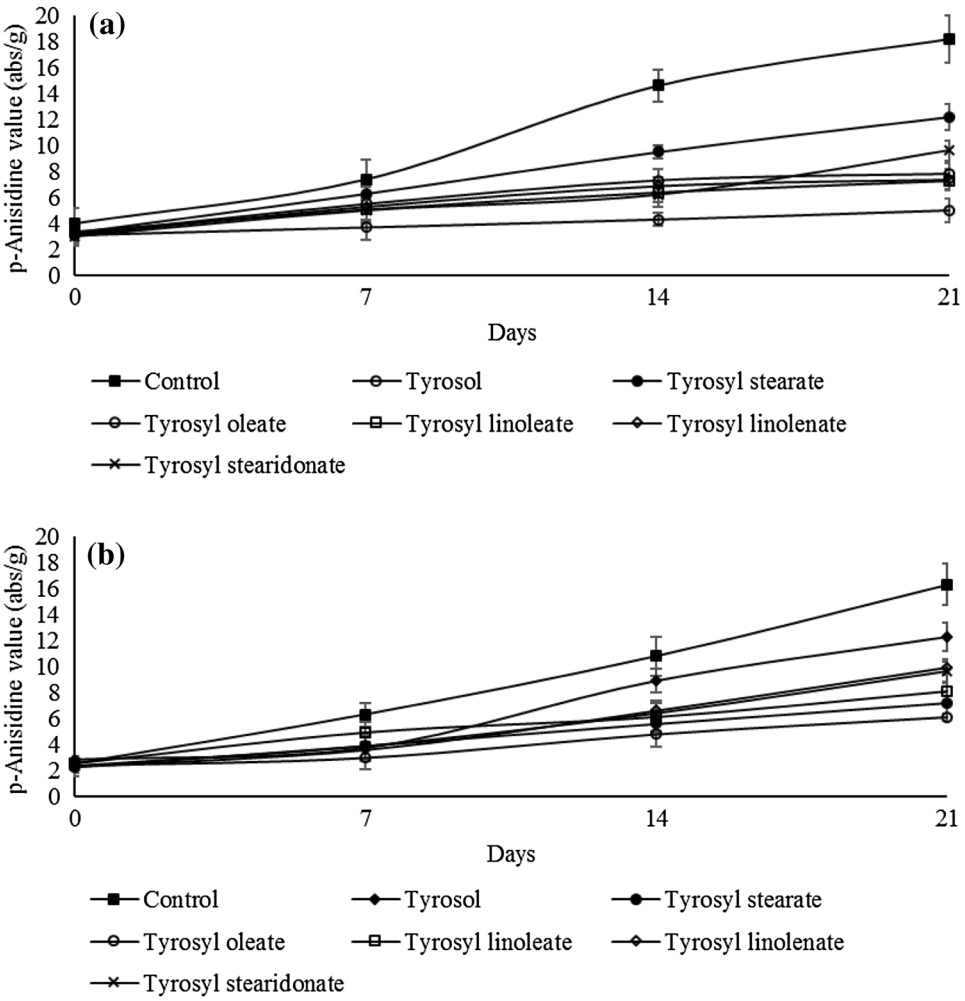


Fig. 5 Effect of lipophilic tyrosol esters on the oxidative stability of **a** structured lipid (SL) and **b** SL-based infant formula emulsion measured by *p*-anisidine value over time at 45 °C. Data points represent the average of duplicate samples

(3.71 mol%) [21]. PV (expressed as mequiv/kg) of the SL and emulsion are shown in Fig. 4a, b, respectively. PV measures the amount of iodine formed by the reaction of the peroxides with the iodine ion. PV is a measurement of the initial/primary products of oxidation. *p*-AV (expressed as absorbance/mL) of the SL and infant formula emulsion are shown in Fig. 5a, b, respectively. *p*-AV determines the amount of aldehydes or decay products of the hydroperoxides by reaction of these compounds with *p*-anisidine. It is a measure of the secondary oxidation products. The TOTOX value is an evaluation of both primary and secondary oxidation products used to estimate oxidative deterioration of food lipids.

In SL, tyrosyl esters exhibited lower antioxidant effectiveness than tyrosol whereas the addition of an alkyl chain enhanced the antioxidant efficiency of tyrosol in infant formula. The PV and *p*-AV of tyrosol for SL after 21 days of storage were 4.9 ± 0.8 and 5.1 ± 0.9 mequiv/kg, respectively. Tyrosol being the most polar was effective in bulk oil system following the polar paradox hypothesis. However, there was no specific order based on polarity for the antioxidant efficiency of the tyrosyl esters. The results indicate that the antioxidant efficiency of a compound is not based only on its polarity as stated in the polar paradox hypothesis but also depends on other factors such as the medium and concentration. The critical site of oxidation in bulk oil is not the air-oil interface rather the association colloids formed with traces of water and surface active molecules such as phospholipids [20]. In another study comparing lipophilic tyrosyl and hydroxytyrosol esters, higher antioxidant capacity was found in hydroxytyrosol due to its *o*-diphenolic structure [10]. In lipid matrix, lower antioxidant activity was reported for the esters than the parent phenol. However, in FRAP and ABTS assays, hydroxytyrosol esters were more efficient than hydroxytyrosol but tyrosol esters were less active than free tyrosol. They also found no significant effect of chain length on antioxidant activity [10]. Similarly, for oil-in-water emulsion (infant formula), a nonlinear trend was observed for the lipophilic tyrosol esters. After 21 days of storage, tyrosyl oleate had the lowest PV (4.3 ± 0.3) and *p*-AV (6.1 ± 0.8) in the emulsion system. Based on the

total oxidation value (TOTOX) the order of antioxidant efficiency in SL was tyrosol (14.8 ± 0.2a), tyrosyl linolenate (20.8 ± 1.1b), tyrosyl oleate (22.6 ± 0.7bc), tyrosyl linoleate (24.1 ± 0.9c), tyrosyl stearidonate (26.0 ± 1.3d), tyrosyl stearate (30.6 ± 1.7e), and control (46.4 ± 2.2f). For the emulsion system, the order was tyrosyl oleate (14.7 ± 0.8a), tyrosyl stearate (17.6 ± 0.9b), tyrosyl linoleate (21.1 ± 1.5c), tyrosyl linolenate (24.7 ± 0.6d), tyrosyl stearidonate (24.8 ± 1.1d), tyrosol (31.9 ± 1.4e), and control (43.1 ± 2.1f). These results show that the most efficient antioxidant in the bulk oil was tyrosol, and in oil-in-water emulsion, tyrosyl oleate was the most efficient antioxidant. Tyrosyl stearidonate was less efficient than tyrosyl oleate and linoleate. This trend may probably be due to complex structure of stearidonate moiety which may cause steric hindrance and hinder the mobility and positioning of the phenolipid on the desired interface in the matrix. It has been postulated that the structure and size of an antioxidant may affect its mobility, location (internalization or interfacial position), and stability (self-aggregation) which in turn determine its antioxidant efficiency in a system [29].

Conclusion

In summary, several lipophilic tyrosyl esters with increasing unsaturation levels were prepared in this study. The oxidation study results from bulk oil and oil-in-water emulsion suggest that the synthesized phenolipids may be used as potential antioxidants in lipid-based products. Addition of an acyl moiety increased the antioxidant activity of tyrosol in the emulsion system. In this study increasing the level of unsaturation in the acyl moiety was not directly proportional to its antioxidant efficiency. Therefore, it is important to determine the best and type of acyl group when synthesizing phenolipids for use as effective antioxidants. Structure modification of tyrosol to increase its lipophilicity may improve its antioxidant effectiveness but further investigations are required to assess the biological activity of these phenolipids.

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