J Am Oil Chem Soc (2010) 87:305–311
DOI 10.1007/s11746-009-1496-y

ORIGINAL PAPER

Enzymatic Synthesis of Novel Feruloylated Lipids and Their Evaluation as Antioxidants

Yan Zheng · Christopher Branford-White · Xiao-Mei Wu · Cheng-Yao Wu · Jian-Gang Xie · Jing Quan · Li-Min Zhu

Received: 4 March 2009 / Revised: 1 October 2009 / Accepted: 16 October 2009 / Published online: 22 January 2010

Abstract Concerns about the use of chemical synthetic antioxidants that promote carcinogenesis has led to the development of natural antioxidants. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid, FA) is a monophenolic phenylpropanoid present in the plant kingdom. It has shown a high antioxidant capacity and biological activities, including antiviral, anticarcinogenic and antimicrobial ones. However, due to poor solubility in hydrophobic media, FA can only be used in aqueous environments and this is a key factor that to a certain extent limits its application and bioavailability. In the present study, novel feruloylated lipids 1(3)-feruloyl-monobutyryl-glycerol (FMB) and 1(3)-feruloyl-dibutyryl-glycerol (FDB) were prepared by lipase-catalysed transesterification between FA and tributyrin. The structure of FMB and FDB was confirmed by NMR and ESI-MS, respectively. The radical scavenging and antioxidant properties of FA, FMB and FDB were evaluated using several different antioxidant assays, including hydroxyl radical scavenging, superoxide anion radical scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radical scavenging, inhibition of peroxidation of linoleic acid and reducing power. The antioxidant activities decreased in the following order: BHT $\geq$ FMB $>$ FDB $>$ FA. The results suggested that FMB and FDB exhibited a strong effect against oxidation in lipophilic systems making them promising antioxidants.

Keywords Enzymatic synthesis · Ferulic acid · Free radical–scavenging activity · Lipophilic antioxidant · Structure–activity relationship

Electronic supplementary material The online version of this article (doi:10.1007/s11746-009-1496-y) contains supplementary material, which is available to authorised users.

Y. Zheng · X.-M. Wu · C.-Y. Wu · J.-G. Xie · J. Quan (✉) · L.-M. Zhu (✉)
College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, 2999 North Renmin Road, 201620 Shanghai, People's Republic of China
e-mail: jquan@dhu.edu.cn

L.-M. Zhu
e-mail: lzhu@dhu.edu.cn

Y. Zheng
e-mail: zhengyan@mail.dhu.edu.cn

C. Branford-White · J.-G. Xie
Institute for Health Research and Policy, London Metropolitan University, London, UK

Introduction

Due to their potential as carcinogens, the demand for commonly used antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) continues to decrease, and so that the development of natural antioxidant has become an area of great interest [1]. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid, FA), one of a number of phenolic compounds, is a natural antioxidant with potential health benefits against cardiovascular problems, inflammatory diseases and certain cancers. FA has long been studied for its health-benefitting properties and its capacity to inhibit oxidation [2]. In addition, the use of FA in pharmaceutical and cosmetic industries and food preparation has also attracted attention [3]. However, due to poor solubility in hydrophobic media FA can only be used in aqueous environments and this is a key factor that limits its application and bioavailability [4].

To overcome this limit, the preparation of lipophilic derivatives of FA through esterification or transesterification of aliphatic molecules is used as a tool to increase its solubility in a hydrophobic media. It has been

demonstrated that the hydrophobic derivatives, such as octyl ferulate, have a higher antioxidant activity [5]. Similarly, triterpene alcohol monoesters such as 24-methylenecycloartenyl ferulate and cycloartenyl ferulate also display significant antioxidant activity and were shown to inhibit oxidation more effectively than FA [6].

The incorporation FA into short-chain triglycerides to produce feruloylated lipids has gained increased interest especially in food applications. This structure provides greater heat stability and emulsifying properties as well as improved biological functions [7, 8]. However, to our knowledge, little effort has been made to investigate the relationship between antioxidant activity and different chemical structures when FA was distributed on the different positions of triglyceride backbone (Fig. 1).

Chemical synthesis of feruloylated lipids is limited due to the heat sensitivity and oxidation susceptibility of FA. In addition, high temperature processes frequently cause a dark color, burnt taste, and high energy consumption [7]. In contrast, a lipase-catalysed reaction has been of great interest because of the relatively lower energy requirement, high substrate specificity and positional selectivity. Moreover, with the steadily growing demand for "natural products", the synthesis of such esters by lipase-catalysed reactions under mild conditions has received much attention [9].

In this study, short-chain structured feruloylated lipids 1(3)-feruloyl-monobutyryl- glycerol (FMB) and 1(3)-feruloyl-dibutyryl-glycerol (FDB) were synthesised by a facile and efficient enzymatic synthesis method. The synthesised compounds structures were identified by NMR and ESI-MS. In order to find the relationship between lipophilic antioxidant activity and different structural features of FMB and FDB, we measured their: (a) OH· radical scavenging; (b) $O_2^{\cdot-}$ radical scavenging activity; (c) DPPH· radical reduction activity; (d) antioxidant activity in linoleic acid emulsion system; (e) reducing power.

Materials and Methods

Materials

Novozym 435 (*Candida antartica* lipase immobilised on polyacrylic resin, with an activity of 10,000 propyl laurate units, PLU/g solid enzyme) was purchased from Novozymes A/S (Bagsvaerd, Denmark). Ferulic acid (FA) (purity >99%) and ethyl ferulate (purity >99%) were purchased from the Suzhou Chang Tong Chemical Co., Ltd (Suzhou, China). TB (purity >99%) was purchased from Tao He Chemical Co., Ltd (Shanghai, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH·), 2,2′-azobis (2-amidinopropane) dihydrochloride (APPH) and linoleic acid, were purchased from the Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. Butylated hydroxytoluene (BHT) was purchased from the Chemical Company, Shanghai, PR China. Other chemicals and solvents in this experiment were all AR grade and from the Shanghai Chemical Reagent Co.

The Enzymatic Reaction

The enzymatic reaction was carried out in a closed, screw-capped tube containing 5 mmol ethyl ferulate, 15 mmol

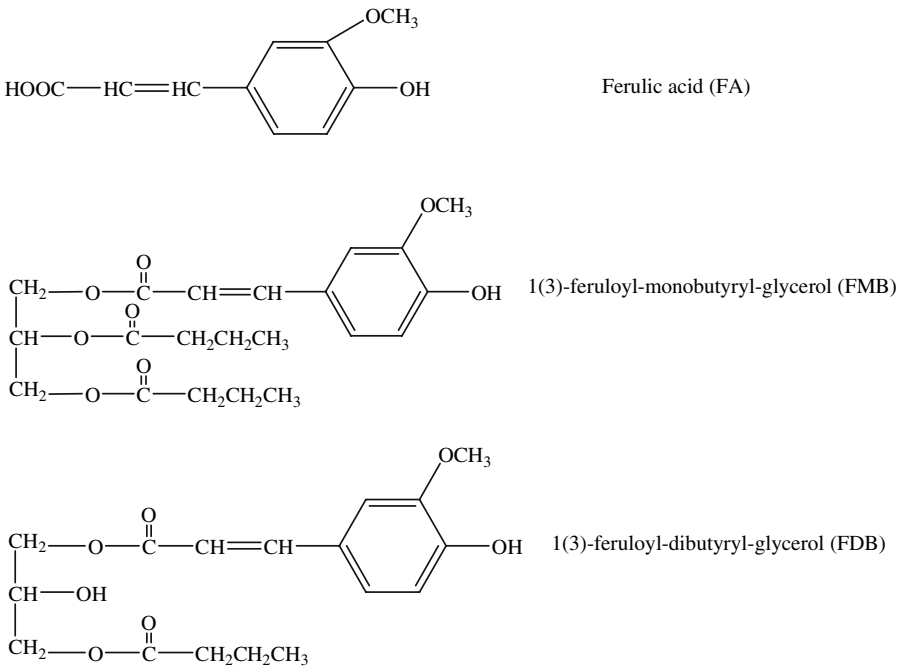

Fig. 1 Structure of ferulic acid (FA), 1(3)-feruloyl-monobutyryl-glycerol (FMB) and 1(3)-feruloyl-dibutyryl-glycerol (FDB)

tributyrin, 360 mg Novozym 435, and 9 ml toluene. The reaction was incubated in an orbital air shaking bath (210 rpm) at 50 °C for 120 h. Control experiments, without enzyme, were carried out in tandem with reactions conducted under identical conditions.

Purification and Identification of the Feruloylated Lipids

Upon the completion of the reaction, the enzyme was filtered off, and the filtrate was concentrated under vacuum. The residue was separated and purified by flash column chromatography using benzene/ether/dichloromethane/hexane (3:5:2:2, v/v/v/v) as the mobile phase. The structures of feruloylated lipids were determined by ^{1}H NMR and ^{13}C NMR (Bruker DRX 400 MHz NMR spectrometer, Germany) at 400 and 100.5 MHz, respectively. $CDCl_3$ was used as the solvent. Chemical shifts were given in ppm relative to TMS as internal standard. The electrospray ionisation-mass spectra (ESI-MS) were recorded on a Shimadzu LCMS-QP 2010 spectrometer.

Identification of FDB

^{1}H NMR ($CDCl_3$, δ, ppm): 7.61 (d, 1H, Ar-H), 7.01–7.30 (m, 2H, Ar-H), 6.90 (d, 1H, =C<u>H</u>-Ar), 6.27 (t, 1H, =CH(CO)), 5.37 (m, 1H, –C<u>H</u>$(CH_2O)_2$), 4.32–4.39 (m, 4H, 2-CH_2O), 3.91 (S, 3H, –OCH_3), 2.31–2.35 (m, 4H, 2-C<u>H</u>$_2$(CH_2CH_3)), 1.63–1.69 (m, 4H, 2-C<u>H</u>$_2$$CH_3$), 0.93–0.97 (m, 6H, 2-$CH_3$). ^{13}C NMR ($CDCl_3$, δ, ppm): 175.5 (–CH_2O<u>C</u>O–), 175.1 (–CHO<u>C</u>O–), 169.0 (–<u>C</u>OCH=), 148.1(=CH–), 150.7, 148.1, 129.0, 125.6, 116.7, 111.9 (Ar), 71.4 (–<u>C</u>H$(CH_2O)_2$), 64.6, 64.5 (2-<u>C</u>H_2O), 58.3 (–OCH_3), 38.4, 38.2 (–<u>C</u>H_2COOCH–, –<u>C</u>$H_2$$COOCH_2$–), 20.7, 20.6 (–<u>C</u>$H_2$$CH_2$COOCH–, –<u>C</u>$H_2$$CH_2$$COOCH_2$–), 15.9,15.8 (2 CH_3–).

ESI-MS (*m*/*z*): 407.9 $[M + H]^+$.

Identification of FMB

^{1}H NMR ($CDCl_3$, δ, ppm): 7.58 (d, 1H, =C<u>H</u>-Ar), 6.84–7.02 (m, 3H, Ar-H), 6.24 (d, 1H, –CH=), 5.86 (m, 1H, –C<u>H</u>$(CH_2O)_2$), 3.83–4.24 (m, 4H, 2-CH_2O–), 3.86 (S, 3H, –OCH_3), 3.82 (d, 1H, –C<u>H</u>(OH)–), 2.28 (t, 2H, –C<u>H</u>$_2$(CH_2CH_3)), 1.58–1.63 (m, 4H, 2-C<u>H</u>$_2$$CH_3$)), 0.89–0.91 (m, 3H, –$CH_3$). ^{13}C NMR ($CDCl_3$, δ, ppm): 176.1 (–<u>C</u>OCH_2–), 169.6 (–CO<u>C</u>H=), 148.2(=CH–), 150.6, 149.1, 129.1, 125.7, 116.8, 111.8 (Ar), 117.1 (–CH=), 70.9 (–CHOH–), 67.6, 67.5(–CH_2O–), 58.3(–OCH_3), 38.3 (–<u>C</u>H_2CO–), 20.7 (–CH_2–), 15.9 (–CH_3).

ESI-MS (*m*/*z*): 337.9 $[M + H]^+$.

log *P* Solubility Measurement

The logarithm of the partition coefficient (log *P*) for *n*-octanol/water was computed using CS ChemPropPro software, an add-on program to ChemDraw Ultra (Cambridge Soft). The program performs the calculations in three different methods using least-squares analysis. The differences of the three methods are based on the number of atomic contributions, the number of molecules used in the database, and the types of atoms in the molecules. The correlation coefficients between the calculated and the observed values of the molecules used in these calculations were in the range of 0.945–0.969. The average log *P* values (SD) of our test compounds were as follows: FA (1.32 ± 0.098), FMB (1.93 ± 0.211) and FDB (3.13 ± 0.176).

Determination of OH· Radical Scavenging

The scavenging abilities of the test compounds for OH· were determined using the deoxyribose assay as described by Halliwell with some modifications [10]. Briefly, the reaction mixture (1.0 ml) containing phosphate buffer (KH_2PO_4–KOH, 0.4 ml, 50 mM pH 7.5), H_2O_2 (0.1 ml, 10 mM), ferric chloride (0.1 ml, 5 mM), ascorbate (0.1 ml, 5 mM), deoxyribose (0.1 ml, 20 mM) and EDTA (0.1 ml, 1 mM), and different concentrations of the test compounds (0.1 ml) were incubated at 37 °C for 1 h. The degree of deoxyribose oxidation was analysed as TBA-reactive material. The absorbance of the reaction mixtures at 532 nm was measured. Decreased absorbance of the mixture indicated scavenging ability and the scavenging percent was calculated as follows:

$$\text{Scavenging percent } (\%) = \frac{Ao - (As - Ac)}{Ao} \times 100\%$$

where *As* was the presence of deoxyribose with test compounds; *Ao* was the presence of deoxyribose without test compounds; and *Ac* was the presence of test compounds without deoxyribose.

Determination of $O_2^{\cdot-}$ Radical Scavenging Activity

$O_2^{\cdot-}$ scavenging capabilities were assayed according to the method previously described by Juliano et al. [11] with some modifications. Reaction mixtures containing Tris–HCl buffer (4.50 ml, 50 mM, pH 8.2 and test compounds (2.0 mg/ml) was incubated at 25 °C for 10 min, and then 300 ml of pyrogallic acid (3 mM, prepared in 10 mM HCl) was added. The absorbance of the reaction mixture at 325 nm was measured immediately at 30-s intervals. The auto-oxidation rate constant (Kb) of pyrogallic acid was calculated from the curve of A_{325} nm versus time. The

inhibitory actions of test compounds against the auto-oxidation rate indicates their $O_2^{\cdot-}$ scavenging abilities.

Determination of DPPH· Radical Reduction Activity

DPPH· scavenging activities of FA, FMB and FDB were carried out as described by Pyo et al. [12] with minor modifications. 0.2 ml of different concentrations (0.02, 0.05, 0.1, 0.2, 0.5, 1 mg/ml) of samples in ethanol were added to 2.0 ml of 1×10^{-4} M ethanol solution of DPPH·. The absorbance at 517 nm was measured after the solution had been allowed to stand in the dark for 60 min. Lower absorbance of the reaction mixture indicates a higher DPPH· scavenging activity. The DPPH· scavenging activity was calculated using the following formula:

$$\text{DPPH}\cdot \text{ scavenging activity}(\%) = \frac{1-(S-SB)}{(C-CB)} \times 100\%$$

where *S*, *SB*, *C* and *CB* were the absorbance of the sample, the blank sample (2.0 ml of ethanol plus 0.2 ml of DPPH· solution plus 0.2 ml of ethanol), and the blank control (ethanol), respectively.

Determination of Antioxidant Activity

The antioxidant activity was evaluated by using 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced lipid peroxidation of a Tween-emulsified linoleic acid system and measured by the ferric thiocyanate assay. Briefly, 0.2 ml of distilled water, 0.5 ml of 0.2 M phosphate buffer, pH 7.0 (prepared from stock solutions of NaH_2PO_4 and Na_2HPO_4, 0.2 M each), and 0.5 ml of 0.25% Tween-20 (in buffer solution) were mixed with 0.5 ml of 2.5% (w/v) linoleic acid in ethanol. The mixture was then stirred for 1 min. The peroxidation was initiated by the addition of AAPH solution (0.1 M, 50 µl). The ethanolic solution of antioxidant (100 µM, 0.5 ml) was then added, and the reaction was carried out at 37 °C for 675 min in the dark. The degree of inhibition of oxidation was measured by the ferric thiocyanate method for each interval of 75, 150, 225, 300, and 375 min. To 0.1 ml of peroxidation reaction mixture at each interval, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 2×10^{-2} M freshly prepared $FeCl_2$ (in 3.5% aqueous HCl) were added. Precisely 3 min after addition, the absorbance of the red complex $[Fe(SCN)]^{2+}$ was measured at 500 nm. The control experiment for the assay was prepared in the same manner by mixing all of the chemicals and reagents except the test compound. BHT served as the reference compound.

Determination of Reducing Power

The reducing power of the feruloylated lipids was determined according to the methods of Jayaprakasha et al. [13]. Briefly, a methanolic solution (0.5 ml) of the samples at various concentrations (0.1, 0.2, 0.5 mg/ml) were mixed with phosphate buffer solution (PBS, 2.5 ml, 0.2 M, pH 6.6) and $[K_3Fe(CN)_6]$ (2.5 ml, 1%, w/v). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%, w/v) was added to the mixture, which was then centrifuged at 1,800*g* for 10 min. The upper layer of solution (2.5 ml) was mixed with deionised water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%, w/v), and the absorbance was measured at 700 nm. The reducing power increased with increasing absorbance of the reaction mixture. BHT was also assayed at the same concentration for comparison purpose.

Statistical Analysis

The results were taken as means ± standard deviation ($n = 6$). For all statistical comparisons, the level of significance was set at $p < 0.05$. Analysis of variance and significant differences among means were tested by one-way ANOVA, using SPSS (Version 13.0 for Windows, SPSS Inc., Chicago, IL).

Results

OH· Scavenging

The deoxyribose degradation assay was used to evaluate samples for their ability to scavenge hydroxyl radical. As shown in Fig. 2, all the test compounds in a concentration-dependent manner. FMB and FDB both showed a sharp increase in scavenging ability with its concentration, and both FMB and FDB exhibited stronger OH· radical scavenging activity (84.9 and 78.6%) than FA (63.2%) at a concentration of 4 mg/ml. When compared with the excellent antioxidant α-tocopherol, the OH· radical scavenging ability of FMB was not significantly different.

$O_2^{\cdot-}$ Radical Scavenging Activity

The $O_2^{\cdot-}$ radical is the most common free radical generated in vivo. Pyrogallic acid can auto-oxidise in alkaline conditions to produce $O_2^{\cdot-}$ directly, and the rate constant of this auto-oxidising reaction is dependent on the $O_2^{\cdot-}$ concentration. Having scavenging capability on $O_2^{\cdot-}$, the test compound significantly slowed down this auto-oxidation reaction of pyrogallic acid. As the data in Table 1 indicated, the

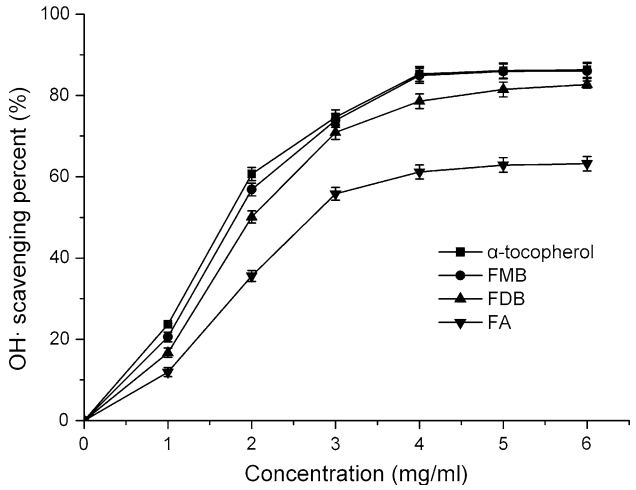

Fig. 2 OH· radical scavenging activity of samples. Values are the means ± SD of sextuple measurements

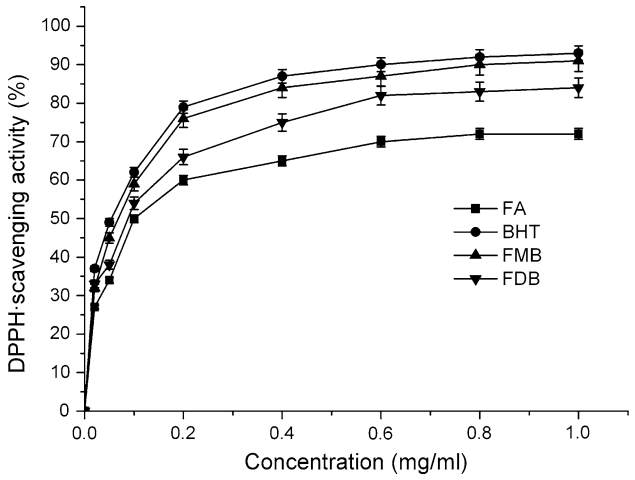

Fig. 3 DPPH· radical scavenging activity of samples. Values are the means ± SD of sextuple measurements. Significant differences are represented as $p < 0.05$

scavenging effect of FMB and FDB was 1.62×10^{-4} and 2.18×10^{-4}, respectively. Both FMB and FDB were more efficient than FA (2.71×10^{-4}), but less than BHT (0.76×10^{-4}) in our experimental conditions.

DPPH· Radical Reduction Activity

Because the DPPH· assay can accommodate many samples in a short period of time and is sensitive enough to detect active ingredients at low concentration, the DPPH· scavenging activity has been widely used to evaluate the antiradical activity of various sample compounds. Figure 3 demonstrates the DPPH· scavenging activities of the FA, FMB, FDB and BHT. As expected, the radical scavenging activity of these compounds increased with increasing the concentration and when the concentration reached 1 mg/ml, BHT, FMB, FDB, and FA scavenged DPPH· at 93, 91, 84 and 72% respectively. It was notable that, at all concentrations investigated, the radical scavenging efficacy of the test samples decreased in the following order BHT > FMB > FDB > FA.

Determination of Antioxidant Activity

The inhibitory effects on lipid peroxidation or the antioxidant activity of the FA, FMB, FDB and BHT are given in Fig. 4. Antioxidant activity is defined as the ability to delay lipid peroxidation according to the method of Son et al. [14]; the time required to reach maximum absorbance of 4. The stronger the antioxidant the longer time it takes to reach this value. As indicated in Fig. 4, the inhibitory activity of the compounds in decreasing order was BHT ≥ FMB > FDB > FA.

Determination of Reducing Power

Previous studies have reported that the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [15]. Thus, it appears necessary to determine the reducing power of the compounds to evaluate their antioxidant potentials. Figure 5 depicts the reducing power of FA, FMB and FDB compared with BHT as a standard using the potassium ferricyanide reduction method. A strong reducing power was noted for compounds BHT and FMB and a lower reducing power was found for compounds FDB and FA. As concentration increased from 0.1 to 1 mg/ml, the reduced power of BHT and FMB increased rapidly from 0.334 and 0.062 to 2.315 and 2.173 respectively, while compounds FDB and FA increased in a moderate way. Overall the reducing power of all samples increased with the concentrations and the reducing power effects of the four compounds were in the following order: BHT > FMB > FDB > FA.

Table 1 Inhibition of the auto-oxidation rate of pyrogallic acid by the test compounds

Test compound	Auto-oxidation	FA	FMB	FDB	BHT
Kb value ($\times 10^{-4}$)	36.21 ± 1.76	2.71 ± 0.22	1.62 ± 0.23	2.18 ± 0.17	0.76 ± 0.12

Data are expressed as mean ± SD ($n = 6$)

* $p < 0.05$, significantly different from the auto-oxidation rate constant

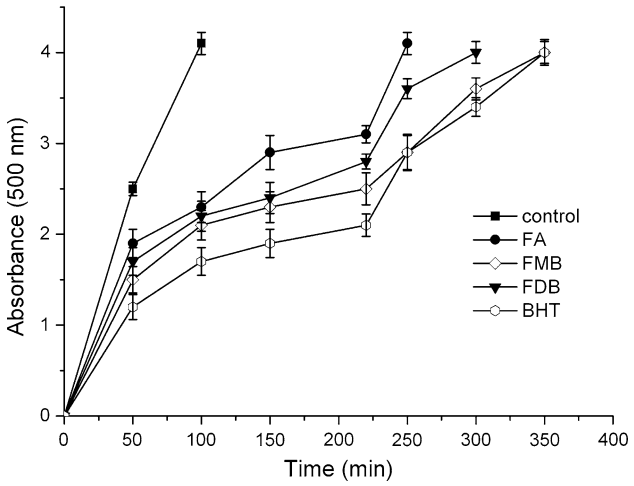

Fig. 4 Antioxidant activity of samples on APPH· in a Tween-emulsified linoleic acid system. Values are the means ± SD of sextuple measurements

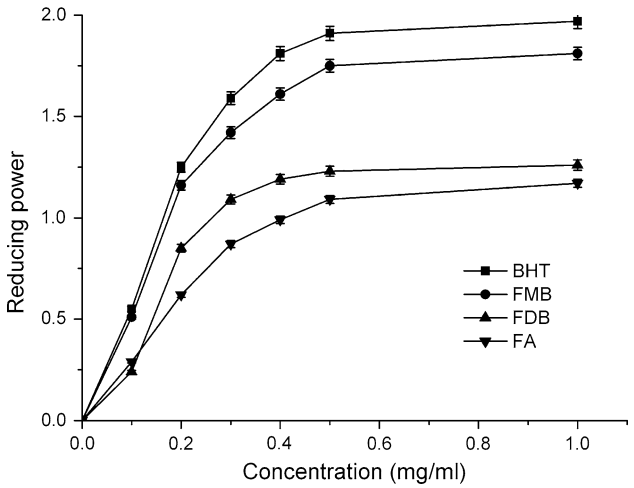

Fig. 5 Reducing power (absorbance at 700 nm) of samples. Values are the means ± SD of sextuple measurements

Discussion

Antioxidants are closely related to their biofunctionalities, such as the reduction of chronic diseases like DNA damage, carcinogenesis and inhibition of pathogenic bacteria growth which is often associated with the termination of free radical propagation in a biological system [12]. Thus, antioxidant capacity is widely used as a parameter for bioactive components.

Since there are multiple ways in which a compound can exert its antioxidant activity, we decided to assess the antioxidant activity of FDB, FMB and FA with several different in vitro models. First of all, we applied experimental models where inorganic oxygen-derived radicals, such as OH· and $O_2^{\cdot-}$, are generated. Then, the effect of them on organic radical was confirmed by their ability in scavenging the lipid-soluble DPPH· radical. The results showed that at all concentrations investigated, both inorganic oxygen-derived and organic radicals scavenging activity of the test samples decreased in the order FMB > FDB > FA. These results demonstrate that structural modification of the FA carboxyl by transesterification with tributyrin could lead to an enhancement of its radical scavenging ability.

It is well known that as one of the phenolic compounds, FA can directly react with radical scavenging and be converted into a much less active product with a quinonic structure [16]. Antioxidant properties of FA stem from the oxidisable *o*-phenolic functionality, which can act as an H-atom donor toward reactive oxygen species and other biological oxidants, as a transition metal chelator preventing Fenton-type processes and as an efficient trap for electrophilic nitrosating agents [17]. Compared to FA, both FMB and FDB have a stronger scavenging activity which could be due to three reasons. Firstly, FMB and FDB also have an *o*-phenolic structure and could be generally oxidised to *o*-quinone by free radicals. Secondly, the substitution of a butyric acid moiety on the carbonyl group of FA residue could result in the delocalisation of the excited electron of the free radical across the entire molecule and thereby stabilising it. The third reason could be due to the conformational change of FA during the transesterification. In other words, FA has a coplanar conformation and upon transesterification of its carboxyl group, the rotation of the phenyl moiety may have been reoriented to a degree that may have led to a conformational change and subsequent orientation towards free radical [1]. The results also indicate that FMB showed a stronger scavenging activity than FDB. It may be attributed to the presence of a hydroxyl in FMB side-chain so enhances H-donating ability of derivatives and subsequent radical stabilisation compared to FDB.

The in vitro model using AAPH-induced lipid peroxidation of Tween-emulsified linoleic acid is a common method used to measure the antioxidant activity of synthetic and natural antioxidants. In this assay, the oxidation is carried out under conditions relatively similar to the in vivo system, and the oxidation rate is proportional to the concentration of AAPH. The reducing power of FA, FMB and FDB was in the order of FMB > FDB > FA. The results show that the antioxidant activity in the emulsion system depends on not only the number of hydroxyl groups but also the hydrophobicity or the partition coefficient (log *P*) and stability of the compounds. On the basis of log *P* values and the results shown in Fig. 4, both FMB (log $P = 1.93$) and FDB (log $P = 3.13$) have stronger antioxidant activity than unmodified FA (log $P = 1.32$). This result is in agreement with the general reports that the hydrophobic derivatives have a higher antioxidant activity

than unmodified FA in lipid emulsions [18]. However, when comparing two compounds of comparable lipophilicity, the structural factors could dominate in determining the activity of a specific compound. Although FMB has a lower log *P* value, it still exhibited stronger antioxidant activity than FDB, which might be due to an additional hydroxyl group of FMB in its structure.

The reducing power of test samples decreased in the following order: FMB > FDB > FA, which was similar to the observation from the antioxidant activity. These results suggest that the antioxidant activities of these compounds were probably due to their reducing capacities. In other words these compounds are acting as electron donors and can thus reduce the oxidised intermediates of lipid peroxidation process, so acting as primary antioxidants and therefore inhibit lipid peroxidation [19].

To conclude, the above assays are based on different aspects of the antioxidant mechanism. The data reported in this study indicate that the different chemical structures of feruloylated lipids are effective antioxidants capable of inhibiting peroxidation and scavenge free radicals. Moreover, both FMB and FDB possess many other biological activities (e. g., sunscreen, antimicrobial, emulsification), which make them useful multifunctional ingredients for pharmaceutical, food and cosmetic formulations. Hence, the strong free radical scavenging action and protective effect against lipoperoxidation of FMB and FDB could make them good candidates for use as natural antioxidant of technological values.

Acknowledgments This work was financially supported by the National Nature Science Foundation of China (No. 50773009); Biomedical Textile Materials "111 Project" from the Ministry of Education of the P.R. China (No. B07024); Grant 08JC1400600 of Science and Technology Commission of Shanghai Municipality.

References

1. Karboune S, Saafari M, Lue BM, Yeboah FK, Kermasha S (2005) Lipase-catalyzed biosynthesis of cinnamoylated lipids in a selected organic solvent medium. J Biotechnol 119:281–290
2. Castelluccio C, Bolwell GP, Gerrish C, Rice-Evans C (1996) Differential distribution of ferulic acid to the major plasma constituents in relation to its potential as an antioxidant. Biochem J 316:691–694
3. Lin FH, Lin JY, Gupta RD, Tournas JA, Burch JA, Selim MA, Monteiro-Riviere NA, Grichnik JM, Zielinski J, Pinnell SR (2005) Ferulic acid stabilizes a solution of vitamins C and E and doubles its photoprotection of skin. J Invest Dermatol 125:826–832
4. Stamatis H, Sereti V, Kolisis FN (2001) Enzymatic synthesis of hydrophilic and hydrophobic derivatives of natural phenolic acids in organic media. J Mol Catal B: Enzym 11:323–328
5. Fang X, Shima M, Kadota M, Tsuno T, Adachi S (2006) Suppressive effect of alkyl ferulate on the oxidation of microencapsulated linoleic acid. Eur J Lipid Sci Technol 108:97–102
6. Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H (2002) Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem 50:2161–2168
7. Zheng Y, Quan J, Zhu LM, Jiang B, Nie HL (2008) Optimization of selective lipase-catalyzed feruloylated monoacylglycerols by response surface methodology. J Am Oil Chem Soc 85:635–639
8. Zheng Y, Quan J, Ning X, Zhu LM (2008) Enhancement in synthesis of novel feruloyl lipids (feruloyl butyryl glycerides) by enzymatic biotransformation using response surface methodology. J Agric Food Chem 56:11493–11498
9. Chang SW, Shaw JF, Shieh CH, Shieh CJ (2006) Optimal formation of hexyl laurate by lipozyme IM-77 in solvent-free system. J Agric Food Chem 54:7125–7129
10. Halliwell B, Gutteridge JMC, Aruoma OI (1987) The deoxyribose method: a simple "test-tube" assay for determination of rate contents for reactions of hydroxyl radicals. Anal Biochem 165:215–219
11. Juliano C, Cossu M, Alamanni MC, Piu L (2005) Antioxidant activity of gamma-oryzanol: mechanism of action and its effect on oxidative stability of pharmaceutical oils. Int J Pharm 299:146–154
12. Pyo YH, Lee TC, Logendral L, Rosen RT (2004) Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies *cycla*) extracts. Food Chem 85:19–26
13. Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. Food Chem 73:285–290
14. Son S, Lobowsky EB, Lewis BA (2001) Caffeic acid phenethyl ester (CAPE): synthesis and X-ray crystallographic analysis. Chem Pharm Bull 49:236–238
15. Jeong SM, Kim SY, Kim DR, Jo SC, Nam KC, Ahn DU, Lee SC (2004) Effect of heat treatment on the antioxidant activity of extracts from citrus peels. J Agric Food Chem 52:3389–3393
16. Milic BLJ, Djilas SM, Canadanovic-Brunet JM (1998) Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system. Food Chem 61:443–447
17. Napolitano A, d'Ischia M (2002) New insights into the acid-promoted reaction of caffeic acid and its esters with nitrite: decarboxylation drives chain nitrosation pathways toward novel oxime derivatives and oxidation/fragmentation products thereof. J Org Chem 67:803–810
18. Weitkamp P, Vosmann K, Weber N (2006) Highly efficient preparation of lipophilic hydroxycinnamates by solvent-free lipase-catalyzed transesterification. J Agric Food Chem 54:7062–7068
19. Ordonez AAL, Gomez JD, Vattuone MA, lsla MI (2006) Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem 97:452–458