GRASAS Y ACEITES 71 (2)
April–June 2020, e352
ISSN-L: 0017-3495
https://doi.org/10.3989/gya.0226191

Butylated methyl caffeate: a novel antioxidant

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Submitted: 20 February 2019; Accepted: 15 April 2019; Published online: 17 June 2020

SUMMARY: A novel caffeic acid derivative, butylated methyl caffeate (BMC), was synthesized *via* esterification between butylated caffeic acid (BCA) and methanol. Its antioxidant activity was investigated and compared to TBHQ, caffeic acid (CA), methyl caffeate (MC) and BCA through deep-frying, an oven test in oil-in-water emulsions and DPPH radical scavenging. BMC showed the strongest antioxidant activity among the five antioxidants in emulsions and its antioxidant activity was almost as strong as BCA in frying. Its soybean oil-water partition coefficient was 9.18 due to its ester and *tert*-butyl groups, far greater than those of MC (4.82), BCA (2.41), CA (0.84) and TBHQ (3.22). This meant that it was much more soluble in the lipid phase than the other four antioxidants in emulsions. The DPPH radical scavenging activity of BMC was near TBHQ, lower than the other three because of its steric hindrance and less functional phenolic hydroxyl groups compared to others when their masses were the same.

KEYWORDS*: Antioxidant activity; Butylated methyl caffeate; Caffeic acid derivatives; Free radical scavenging; Frying and emulsions*

RESUMEN: ***Cafeato de metilo butilado: un nuevo antioxidante*****.** Un novedoso derivado del ácido cafeico, el cafeato de metilo butilado (BMC), fue sintetizado mediante esterificación entre el ácido cafeico butilado (BCA) y el metanol. Se investigó su actividad antioxidante y se comparó con TBHQ, ácido cafeico (CA), cafeato de metilo (MC) y BCA mediante pruebas de fritura, pruebas en horno, en emulsiones de aceite en agua y mediante eliminación de radicales DPPH. BMC mostró la mayor actividad antioxidante entre los cinco antioxidantes en emulsiones y tenía una actividad antioxidante casi tan fuerte como la del BCA en fritura. Su coeficiente de partición aceite de soja-agua es de 9.18 debido a sus grupos éster y *terc*-butilo, mucho mayores que los de MC (4.82), BCA (2.41), CA (0.84) y TBHQ (3.22). Esto significa que es mucho más soluble en la fase lipídica que los otros cuatro antioxidantes cuando está en emulsiones. La actividad de captación de radicales DPPH de BMC fue cercana a la del TBHQ, más baja que las otras tres debido a su impedimento estérico y grupos hidroxilo fenólicos, menos funcionales en comparación con los otros cuando sus masas son iguales.

PALABRAS CLAVE: *Actividad antioxidante; Cafeato de metilo butilado; Derivados del ácido cafeico; Eliminación de radicales libres; Fritura y emulsiones*

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Citation/Cómo citar este artículo: Chen QQ, Pasdar H, Weng XC. 2020. Butylated methyl caffeate: a novel antioxidant. *Grasas Aceites* **71** (2), e352. https://doi.org/10.3989/gya.0226191

1. INTRODUCTION

The autoxidation of lipids produces peroxides and then forms small, volatile compounds which produce the off-aromas which damage food quality and harm human health. The addition of antioxidants is the most common and economical method to retard autoxidation. In addition to some common antioxidant activity assays, such as the hydroperoxide test, the Rancimat test, the thiobarbituric acid reactive species assay *etc.* (Amorati and Valgimigli, 2014; Cui *et al.*, 2015), more and more new technologies are being used for the detection of antioxidant activities in foods, including headspace gas chromatography, adding a molecular probe to the oxidizable substrate, conjugated autoxidizable triene and the apolar radical-initiated conjugated autoxidizable triene assays, *etc.* (Haidasz *et al.*, 2016; Homma *et al.*, 2015; Phonsatta *et al.*, 2017). Furthermore, most foods are multiphase systems, in which lipids are usually present in emulsions (Mcclements and Decker, 2000). The efficacy of antioxidants would be influenced by their location in emulsion systems due to their polarity and solubility, which have different situations in bulk oil systems (Lisete-Torres *et al.*, 2012; Phonsatta *et al.*, 2017). Therefore, it is necessary to establish different models to investigate the antioxidant activities of antioxidants in lipids.

Caffeic acid (CA, Figure 1), one of the most common antioxidants during the oxidative deterioration of lipids, often occurs in fruits, grains and some traditional Chinese medicinal herbs (Hao *et al.*, 2015). Since the poor solubility of CA in lipids limits its application in fatty foods, more and more focus is placed on caffeic acid derivatives. Esterification modification can be a good way to improve its fat-solubility and antioxidant activities and widen its application. Methyl caffeate (MC, Figure 1) was confirmed as the simplest caffeic acid ester and could effectively improve the cell's protective activity against oxidative stress (Garrido *et al.*, 2012). Jia *et al.*, (2015) found that caffeic acid phenethyl ester could restrain oxidation in soybean oil-in-water emulsions, which was connected with their affinities to lipid droplets in emulsions. In addition, the maximum permitted concentration of synthetic antioxidants added to edible oils is 200 mg/kg according to international regulations (Yue *et al.*, 2016).

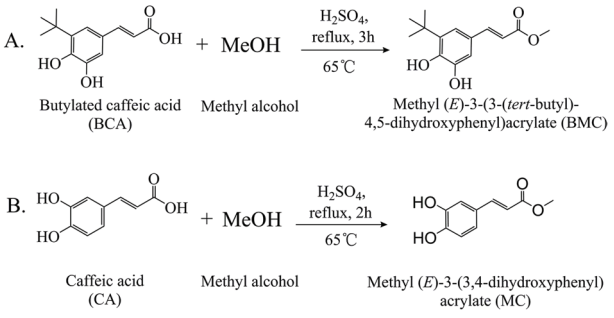


FIGURE 1. A. Esterification of butylated caffeic acid (BCA) to obtain butylated methyl caffeate (BMC); B. Esterification of caffeic acid (CA) to obtain methyl caffeate (MC).

Shi *et al.*, (2017) synthesized butylated caffeic acid (BCA, Figure 1) in a laboratory after adding *tert*-butyl group on the benzene ring and its antioxidant capacity was greatly improved during frying. In this study, a novel caffeic acid ester derivative, butylated methyl caffeate (methyl (*E*)-3-(3-(*tert*-butyl)-4,5-dihydroxyphenyl) acrylate, BMC, Figure 1), was synthesized *via* esterification with an acid catalyst between BCA and methanol. In addition, a bulk oil system and a soybean oil-in-water emulsion system were established to investigate the antioxidant activities of BMC and *tert*-butylhydroquinone (TBHQ), CA, MC, BCA, using a deep frying experiment, oven test and DPPH spectrophotometric assay.

2. MATERIALS AND METHODS

2.1. Chemicals

Commercial soybean oil (acid value: 0.17g KOH/kg; iodine value: 124.7 g/100g) was purchased from the supermarket, Shanghai, China. CA, TBHQ, Tween 80, KH_2PO_4 and $K_2HPO_4.7H_2O$ were purchased from Sigma-Aldrich Trading Co. Ltd. Silica gel and other chemicals used in this experiment were all AR grade and came from Sinopharm Chemical Reagent Co. Ltd. All of the reactions were monitored by thin-layer chromatography (TLC) performed on silica gel GF254.

2.2. Spectra recording

Nuclear magnetic resonance (NMR) spectra were recorded with an Avance 400 MHz spectrometer (Bruker, Switzerland). Ultraviolet (UV) spectra were recorded with a UV-2450 spectroscopic instrument (Shimadzu Corp, Kyoto, Japan). High resolution mass spectrometry analysis (HRMS) was recorded by Thermo Scientific LTQ Orbitrap XL with electron spray ionization (Kee Cloud Biotech, Shanghai, China). High performance liquid chromatography (HPLC) Agilent 1100 was equipped with Agilent ZORBAX Eclipse XDB (250×4.6mm, 5μm) (Agilent Technologies Inc., America). A Zetasizer Nano S09 was bought from Malvern Instruments (Worcestershire, UK).

2.3. Synthesis and purification of the compounds

BCA were prepared according to the method of Shi *et al.*, (2017). A mixture of BCA (0.05 mol, 1.15g) and 30 ml methanol was added in a flask at 65 °C under stirring followed by the dropwise addition of H_2SO_4 (98%, 3ml). After refluxing for

3 hours, the finished reaction mixture was extracted by ethyl acetate (30 ml, 3 times). The organic phase was washed with ultrapure water, a saturated solution of NaCl (36g NaCl/100ml H_2O at room temperature), and dried over Na_2SO_4 and concentrated under reduced pressure. The mixture was separated by column chromatography (CH_2Cl_2/MeOH, 20:1) and the yield of BMC (white needle crystal) was 78%. ^{1}H NMR were (400 MHz, Acetone-d_6) δ 9.01 (s, 1H), 7.67 (s, 1H), 7.51 (d, J = 15.9 Hz, 1H), 7.20–6.88 (m, 2H), 6.20 (d, J = 15.9 Hz, 1H), 3.68 (s, 3H), 1.39 (s, 9H). ^{13}C NMR were (100 MHz, Acetone-d_6) δ 169.77, 149.75, 148.34, 147.54, 138.69, 127.75, 122.86, 116.58, 114.04, 53.36, 37.14, 31.57. The data conformed with the spectra data reported by Shi *et al*., (2017). HRMS (ESI): m/z was calculated for $C_{14}H_{19}O_3^+$ $(M+H)^+$ 235.13287, and found to be 235.13303.

The MC was prepared with the same method, and pale yellow powder crystals were obtained (91%). ^{1}H NMR were (400 MHz, Acetone-d_6) δ 7.55 (d, J = 15.9 Hz, 1H), 7.18 (d, J = 2.1 Hz, 1H), 7.05 (dd, J_1 = 8.2 Hz, J_2 = 2.1 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.30 (d, J = 15.9 Hz, 1H), 3.73 (s, 3H), and conformed with the spectra data for MC as Masuda (2008) reported.

2.4. Antioxidant activity during deep frying

The purchased soybean oil was purified in a 100–200 mesh silica gel column to remove endogenous and exogenous antioxidants (especially tocopherols) according to the method by Samdani *et al*., (2018) with a slight modification. The removal of tocopherols in the soybean oil was verified by HPLC (Kiralan *et al.*, 2014). 0.02% of different antioxidants were added to purified soybean oil (500g) and it was heated to 180 ± 5 °C. About 30 ± 5g potato chips (3mm) were added into the oil every hour and removed after frying for 8 min. Oil samples were taken at regular intervals. After 15 h, the oil was cooled and kept at room temperature for one night. This procedure was repeated twice. Acid values (AV), Iodine values (IV) and Conjugated diene (CD) values were determined for samples using the International Union of Pure and Applied Chemistry (IUPAC) method (Paquot, 1979; Okubanjo *et al.*, 2019).

2.5. Emulsion preparation and oven test

Soybean oil-in-water emulsions (500 g) were prepared as mixtures of purified soybean oils, Tween 80 and phosphate buffer solution (pH 7.0). Tween 80 was used as an emulsifying agent. These samples were divided into experimental groups (0.02% antioxidants added) and a blank group (no antioxidant added). The purified soybean oils, Tween 80 and phosphate buffer solution were mixed in 500 ml Erlenmeyer flasks at the ratio of 2:1:7 (25 °C). The emulsions were made after shaking vigorously for 30 min and sonicating in an ice bath for 15 min. The whole system used a pH meter to ensure pH at 7. The emulsions were visually stable for at least 12 h and stored at room temperature in the dark. Particle size distributions of the emulsions were measured by Zetasizer Nano S09 (triplicate, room temperature) according to the method of Kiralan *et al*., (2014). The average emulsion droplet size was 499.0 ± 38.1 nm, similar to Okubanjo *et al*., (2019), and there was no significant visual change of any emulsion state over the course of the oven test.

The emulsions were held in an oven at 63 ± 1 °C for 35 days and the peroxide values (POV) were measured as the index according to the American Oil Chemists Society (AOCS) Official Method Cd 8–53 once a day (Firestone, 1998). The POV were calculated by the following equation (Firestone, 1998):

$$\mathrm{POV(meq/kg)} = \frac{(V - V_0) \times N}{m} \times 1000$$

Where, V is the volume (ml) of sodium thiosulfate solution consumed by the emulsion sample, V_0 is the volume (ml) of sodium thiosulfate consumed by the blank sample, N is the normality of the sodium thiosulfate solution, and m is the weight of the emulsion sample (g).

2.6. Soybean oil-water partition coefficients of antioxidants in emulsions

The experiment was based on the spectral properties of different antioxidants. Five antioxidant absorbance spectra from 200 to 500 nm were determined to determine their characteristic absorption peaks (λs). Then, antioxidant absorbance values of a series of concentrations were tested at λs to get linear regression equations where the absorbance values (nm) were plotted against concentrations. The emulsions prepared by the oven test method were broken with a saturated solution of NaCl and centrifuged (8000 r/min, 10min, 3 times). The water phases dissolved by ethanol and their absorbance were tested to determine the solubility of antioxidants in water. The soybean oil-water partition coefficients (SPC) of the antioxidants in emulsions were calculated by the following equation (Sinadinovic-Fiser and Jankovic, 2007):

$$\mathrm{SPC} = \frac{S_A^o}{S_A^w}$$

Where, S_A^i (μg/ml) is the solubility of antioxidants (A) in phase i (o-oil, w-water).

2.7. Antioxidant activity evaluated by DPPH spectrophotometric assay

The DPPH method was used to determine the antioxidant activities of BMC, MC, CA, BCA and TBHQ. The radical scavenging activity of the different samples was measured according to the method by Liu *et al*., (2017). All spectrophotometric measurements were carried out with a UV-2450 spectroscopic instrument (Shimadzu Corp, Kyoto, Japan). EC50, the concentration that causes a decrease in the initial DPPH concentration by 50% (Sanchez-Moreno *et al.*, 1998), was calculated by linear regression of plots where the radical scavenging activity (%) was plotted against concentration. DPPH radical scavenging activity was calculated from the following equation:

$$\text{Scavenging activity} = 1 - \frac{(A_{sample} - A_{blank})}{A_{control}} \times 100\%$$

Where, A_{sample} are DPPH solutions (2.5 ml) with different concentrations of antioxidant solution (0.5 ml), A_{blank} are different concentrations of antioxidant solution (2.5 ml) with methanol (0.5 ml), $A_{control}$ is the DPPH solution (2.5 ml) with methanol (0.5 ml).

2.8. Statistical analysis

The results were expressed as mean ± standard deviation (SD) of experiments for the analytical determination. Statistical significance between different groups was determined by the analysis of variance (ANOVA) using IBM SPSS Statistics 22, followed by Duncan's multiple comparisons test ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Structure of the new compound

BMC (MC) was obtained from esterification between BCA (CA) and methanol as shown in Figure 1. Rf values of BMC and BCA obtained by thin-layer chromatography (TLC) were 0.67 and 0.01, respectively (dichloromethane/methanol, 20:1). BMC had a characteristic UV absorption peak at 334 nm (0.658). After adding KOH solution to the BMC solution, the absorption peak showed a red shift to 383 nm (0.566) which confirms the presence of phenolic hydroxyl groups on BMC. All NMR and MS data confirmed the structure of BMC as shown in Figure 1.

3.2. Antioxidant activity during deep frying

The deep-frying technique includes the dipping or contacting of foods with hot oils at the temperature range of 170–190 °C to increase their organoleptic qualities and shelf lives (Oreopoulou *et al.*, 2006). At such a high temperature (180 °C) and prolonged exposure of the oil to oxygen make an additional demand on antioxidants during frying such as thermally stable low volatility (Catel *et al.*, 2010). At the same time, lipids are easily oxidized and deteriorated to result in off-flavors at such a high temperature, which can be measured to evaluate the effect of added antioxidants.

Free fatty acid content increased because of the oxidative rancidity and hydrolysis of glycerides during deep frying (Hammouda *et al.*, 2018). Changes in the AV of oils during deep frying are shown at Figure 2A. At first, the AV of the Blank sample was 0.17 g KOH/kg. After time, the AV of the Blank sample had obviously increased and its AV was

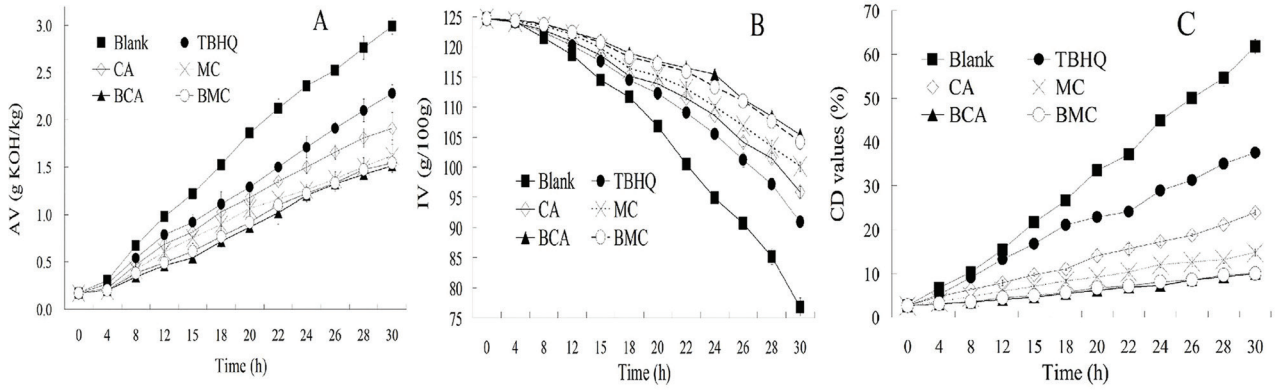


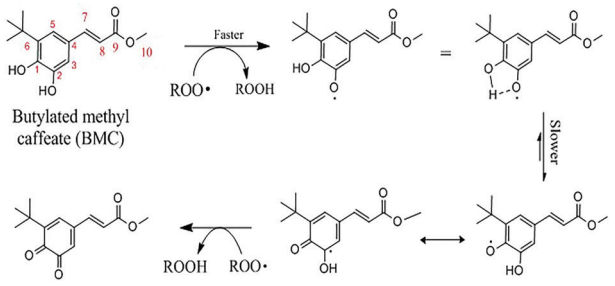
FIGURE 2. A. Changes in acid values (AV) of different compounds against time at a concentration of 0.02% at 180 ± 5 °C in soybean oils; B. Changes in iodine values (IV) of different compounds against time at a concentration 0.02% at 180 ± 5 °C in soybean oils; C. Changes in the conjugated diene (CD) values of different compounds against time at a concentration of 0.02% under 180 ± 5 °C in soybean oils (Mean ± SD, n = 2) with statistical significance at $p < 0.05$.

2.99 g KOH/kg after 30 h frying, far higher than the samples with added antioxidants. After 30 h frying, BMC showed a high antioxidant activity. Its AV was 1.54 g KOH/kg, which was near BCA (1.51 g KOH/kg) and MC (1.62 g KOH/kg) ($P > 0.05$), lower than CA (1.91 g KOH/kg) and TBHQ (2.28 g KOH/kg) ($P < 0.05$). The changes in AV (Figure 2A) manifest the capacity of antioxidants as follows: BCA ≈ BMC ≈ MC > CA > TBHQ > Blank.

Iodine values (IV) are often used to determine the degree of unsaturation in fatty acids. The higher the IV, the more unsaturated fatty acid bonds are present in the fat (Choudhary *et al.*, 2015). Changes in the IV of oils during deep frying are shown at Figure 2B. It was found that the IV of the Blank sample was 124.7 g/100g under fresh conditions. Its IV decreased rapidly during deep frying and was 76.8 g/100g at the end of the experiment. The IV of the BMC-added sample changed slightly and its IV was 104.2 g/100 g after 30 h frying, which was similar to the BCA-added one (105.4 g/100 g) ($P > 0.05$), better than the MC-added sample (100.1 g/100 g), CA-added (95.9 g/100 g) and TBHQ-added (91.0 g/100 g) ($P < 0.05$). The changes in AV (Figure 2B) manifest the capacity of antioxidants as follows: BCA ≈ BMC > MC > CA > TBHQ > Blank.

During deep frying, there is a shift in the double bond positions of poly-unsaturated fatty acids in soybean oil due to isomerization and conjugate double bond formation, accompanied by increased UV absorption at 233 nm for conjugated diene (CD) values (Okubanjo *et al.*, 2019). Changes in the CD values of oils during deep frying are shown at Figure 2C. At first, the CD value of the Blank sample was 2.61% and at the end of deep frying it was 61.79%. At end of deep frying, the CD value of the BMC-added sample BMC (10.01%) was similar to that of BCA (9.98%) ($P > 0.05$), lower than that of MC, CA, TBHQ, which were 14.82, 23.99, and 37.51%, respectively ($P < 0.05$). It seemed that BMC could effectively prevent the oxidation of oil under frying conditions. The percentage changes in the CD values (Figure 2C) indicated that the antioxidant activities decreased in the following order: BCA ≈ BMC > MC > CA > TBHQ > Blank.

All in all, BMC exhibited a stronger antioxidant activity than TBHQ, CA and MC during deep frying, similar to BCA, indicating that it was effective under high temperature conditions, thermally stable and retained low volatility to prevent evaporation (Shi *et al.*, 2017). The proposed antioxidant mechanism can be seen in Scheme 1. The *tert*-butyl attached to the 6-position of BMC (Scheme 1) is a very large-sized group, which exerted a strong steric hindrance. Due to the crowded environment, the 2-position hydroxyl of BMC donates the hydrogen atom easily to active radicals and two hydroxyls have a much stronger spatial synergy to form a stable intermediate when a hydrogen atom leaves



SCHEME 1. The proposed antioxidant mechanism of butylated methyl caffeate (BMC).

(Boilet *et al.*, 2005). Hydroxycinnamoyl structures enlarge the resonance system and make the positive charge density of the carbon atom attached to hydroxyl in the 1-position of BMC reduced, which is favorable for the formation of a stable dehydrogenated semi-quinone radical (Moon and Terao, 1998). Finally, BMC captures two radicals on two phenolic groups to produce an *o*-quinone (Scheme 1). Meanwhile, the ester group increases the hydrophobicity and fat solubility of BMC, making it easier work with.

3.3. Antioxidant activity in the oven test

POV is normally used as the detection of the initial stages of the development of rancidity in lipids combined with the oven test since hydroperoxides are the primary products of lipid oxidation and fall in the latter stage of oxidation due to decomposition into secondary products (aldehydes, ketones, *etc.*) after reaching a maximum (Gray, 1978; Cisneros *et al.*, 2014). The POV of all the samples increased continuously during the oven test at 63 °C for 35 days (Figure 3). The POV of the Blank sample was 4.6 meqO_2/kg on the first day. It grew steadily at the beginning of the experiment and its growth rate began to accelerate during 18–25 days and rose rapidly after 25 days. It reached a maximum on the 35th day (382.6 meq O_2/kg oil), which meant that the emulsion had been severely rancid. It began to decline after 35 days (353.6 meqO_2/kg oil) and reflected the expected degradation to secondary oxidation products (Cisneros *et al.*, 2014). The changes in the POV of the BMC-, BCA-, MC-, CA- and TBHQ-added samples showed that adding antioxidants was better for preventing the oxidation of emulsions and they were 105.6 meq/kg oil, 218.7 meq O_2/kg oil, 174.6 meq O_2/kg oil, 361.1 meq O_2/kg oil, 260.0 meq 0_2/kg oil on the last day, respectively ($P < 0.05$). This indicated that the antioxidant activities of the antioxidants in emulsions decreased in the following order: BMC > MC > BCA > TBHQ > CA. This result was slightly different from that of deep frying, which may be related to the distribution of antioxidants in the oil-water mixture.

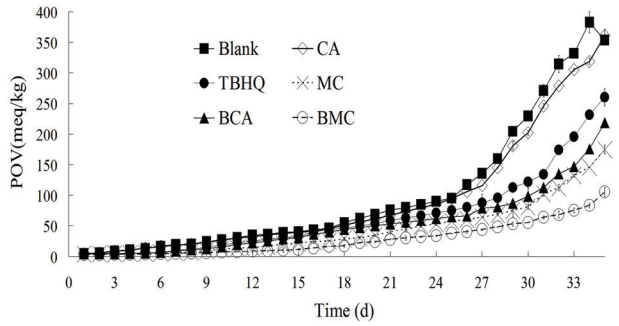


FIGURE 3. The peroxide values (POV) of the emulsion systems during the oven test (63 ± 1 °C). Reported data were expressed as (n = 2) with statistical significance at $p < 0.05$.

3.4. Soybean oil-water partition coefficients of antioxidants in emulsions

To confirm the results of the oven test, the experiment on the distribution of antioxidants in two phases of emulsion was designed. The results of the UV scanning showed that the λs of the five antioxidants were 293 nm (TBHQ), 325 nm (CA), 330 nm (MC), 324 nm (BCA), and 333 nm (BMC), respectively. The solubility of the five antioxidants in water calculated through linear regression equations ($R^2 > 0.98$, Table 1) decreased in the following order: CA >> BCA > TBHQ > MC >> BMC and their SPC in emulsions were 9.18 (BMC), 4.82 (MC), 3.22 (TBHQ), 2.41 (BCA), 0.84 (CA). This meant that BMC was the most lipid-soluble compared to water, followed by MC, TBHQ, BCA and CA.

The whole study indicated that the reason for the difference between results from the deep frying and oven tests was that the ester group overcame the limitations of the intramolecular hydrogen bond and increased the hydrophobicity of BMC and MC, making them more easily dissolved in lipids to function in oil-in-water emulsions. At the same time, with the influence of intramolecular and intermolecular hydrogen bonding, the protonation/deprotonation of the -COOH group, most of the CA were dissolved in water rather than in lipids and so could not work well as antioxidants in emulsions (Pekkarinen *et al.*, 1999). Although the *tert*-butyl attached to benzene ring reduces the polarity of BCA, it is still quite polar and distributes itself into the water phase much due to two phenolic hydroxyl and a carboxyl. BCA worked better than TBHQ in the oven test even if the SPC was lower than TBHQ, which was mainly because the structure of BCA gave itself a much stronger antioxidant activity than TBHQ. This reflects the fact that antioxidant activity is not only related to the distribution of antioxidants in the different phases, but also to their structures (Mcclements and Decker, 2000).

3.5. Antioxidant activity evaluated by the DPPH spectrophotometric assay

The antioxidant activities of BMC, MC, CA, BCA and TBHQ were determined at 517 nm for the free radical-scavenging ability using DPPH. The reaction kinetic between DPPH and antioxidants was investigated through kinetic investigation. The EC_{50} (mg/L) of different antioxidants calculated by linear regressions of plots were as follows: CA^d (0.973) < BCA^c (1.230) < MC^b (2.365) < $TBHQ^a$ (3.584) ≈ BMC^a (3.591) ($P < 0.05$), where different letters meant significantly different using ANOVA followed by Duncan's multiple comparison test. This indicated that the DPPH radical scavenging activities of antioxidants decreased in the following order: CA > BCA > MC > TBHQ ≈ BMC. The reasons for the low DPPH radical scavenging activities of BMC may be as follows: the DPPH is too bulky to be combined with BMC at the steric hindrance influence of *tert*-butyl (Huang *et al.*, 2014); the esterification of BMC lowered its DPPH radical

TABLE 1. The five antioxidant absorbance values of different concentrations at the characteristic absorption peaks and their solubility in water and the soybean oil-water partition coefficients (SPC) of emulsions.

Concentration (μg/ml)	TBHQ	CA	MC	BCA	BMC
10	0.073 ± 0.000	0.122 ± 0.001	0.409 ± 0.000	0.274 ± 0.001	0.116 ± 0.004
20	0.140 ± 0.001	0.277 ± 0.001	0.813 ± 0.003	0.523 ± 0.000	0.361 ± 0.000
30	0.218 ± 0.001	0.525 ± 0.000	1.203 ± 0.001	0.828 ± 0.002	0.652 ± 0.000
40	0.315 ± 0.001	0.864 ± 0.001	1.437 ± 0.003	1.164 ± 0.004	1.010 ± 0.001
50	0.394 ± 0.002	1.028 ± 0.002	1.993 ± 0.000	1.526 ± 0.000	1.313 ± 0.004
60	0.537 ± 0.000	1.258 ± 0.001	2.381 ± 0.001	1.815 ± 0.001	1.769 ± 0.003
Linear regression equation	y = 0.009x - 0.038 R^2 = 0.985	y = 0.024x - 0.148 R^2 = 0.991	y = 0.039x+0.009 R^2= 0.992	y = 0.032x - 0.083 R^2 = 0.997	y = 0.033x - 0.278 R^2 = 0.991
Solubility in water (μg/ml)	24.00	42.04	18.64	28.09	11.58
SPC	3.22	0.84	4.82	2.41	9.18

[a]Reported data were expressed as Mean ± SD (n = 2).

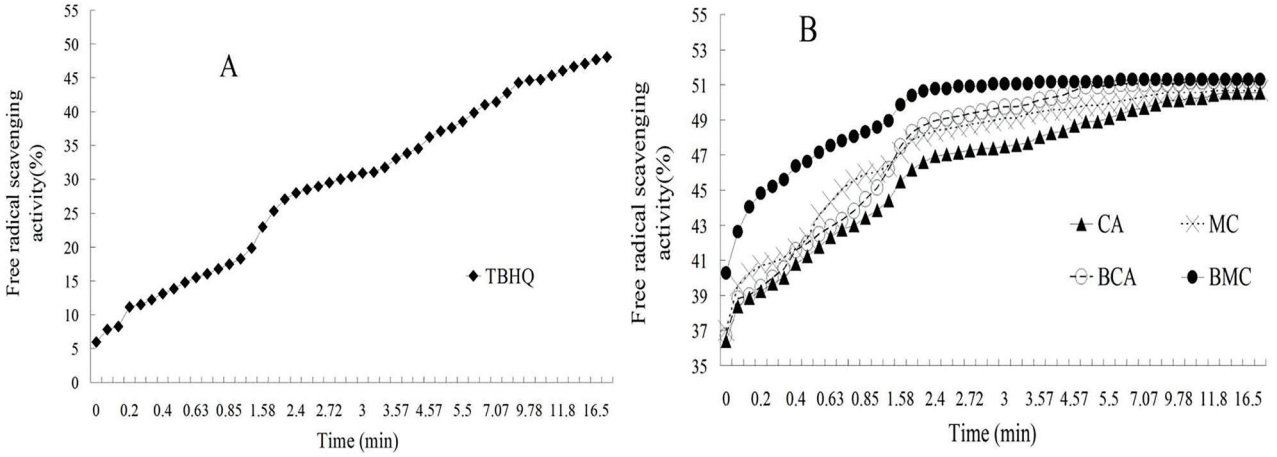


FIGURE 4. The kinetic behaviors of *tert*-butylhydroquinone (TBHQ), caffeic acid (CA), methyl caffeate (MC), butylated caffeic acid (BCA) and butylated methyl caffeate (BMC) at EC_{50} concentration in DPPH.

scavenging activities (SoRensen *et al*., 2014); the number of phenolic hydroxyl groups of BCM that can capture electrons was less than that of the other four antioxidants with the same mass.

The kinetic behaviors of the antioxidants were as shown in Figure 4. According to Sanchez-Moreno *et al*., (1998), the time needed to reach the steady state with EC_{50} was to be determined by the kinetic behavior of the antioxidant, which was classified as follows: < 5 min (rapid), 5–30 min, and > 30 min (slow). So the ease of donating hydrogen (Figure 4 A and B) was BMC (rapid) > BCA (rapid) > MC > CA > TBHQ (slow). The result was a little different from that of the DPPH spectrophotometric assay because sometimes the reaction kinetic between DPPH and antioxidants is not linear to antioxidant concentration or dilution (Karadag *et al.*, 2009).

4. CONCLUSIONS

A novel caffeic acid derivative, BMC, was synthesized *via* esterification and its antioxidant activity was investigated with other four antioxidants in frying and emulsions. BMC showed high antioxidant activity in different systems, especially in emulsions due to its *tert*-butyl and ester groups. The excellent performance of BMC in emulsions makes it more widely used in practical applications. The study provides relevant theoretical basis for the future structural modification of natural antioxidants to expand their application in lipids.

ACKNOWLEDGMENTS

The authors thank Mrs. Y.H. Song of the Center for Supramolecular Materials and Catalysts, Shanghai University for recording and elucidating the NMR spectra, and Mr. G.S. Shi in our group for suggestions.

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