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Antioxidant Activity of Capsaicinoid in Canola Oil

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ABSTRACT: Interest in replacing synthetic antioxidants, namely, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), with natural antioxidants is increasing. The present study examined the antioxidant activity of capsaicinoid from chili pepper in heated canola oil. The oxidation was conducted at 60, 90, 120, and 180 °C by monitoring oxygen consumption and the decrease in linoleic acid and α-linolenic acid in canola oil. At 60 °C, capsaicinoid was more effective against oxidation of canola oil compared with BHT. At higher temperatures of 90, 120, and 180 °C, capsaicinoid possessed an antioxidant activity similar to or slightly weaker that that of BHT. It was found that capsaicinoid prevented canola oil from oxidation in a dose-dependent manner. To study the structure−antioxidant relationship, it was found that the trimethylsiloxy (TMS) derivatives of capsaicinoid did not exhibit any antioxidant activity, suggesting the hydroxyl moiety was the functional group responsible for the antioxidant activity of capsaicinoid. It was concluded that capsaicinoid had the potential to be further explored as a natural antioxidant in foods, particularly spicy foods.

KEYWORDS: *capsaicin, capsaicinoid, lipid oxidation, canola oil*

INTRODUCTION

Capsaicinoid refers to a group of pungent compounds found in chili peppers. Interest in their biological activity is increasing. Previous studies have indicated that red pepper and capsaicinoid decrease blood cholesterol concentration,[1−3] possibly mediated by inhibition of intestinal cholesterol absorption.[4] Capsaicinoid has also been shown to be effective in weight reduction mediated by enhancing β-oxidation of fatty acids in vivo and increasing adrenergic activity and energy expenditure.[5] Accumulated evidence has also demonstrated that capsaicinoid has potential beneficial effect on the human cardiovascular system.[6,7] It has also been reported that capsaicinoid possesses antitumor activity.[8,9]

Lipid oxidation is associated with deterioration of oil quality and is responsible for the rancidity of edible oils. Synthetic antioxidants, namely, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are still widely used as preservatives in foods due to their high thermal stability, low cost, and efficacy, although research has shown that they may be carcinogenic and cytotoxic at a high dose.[10,11] Natural antioxidants are therefore highly desirable because of the general public's rejection of the use of chemically synthesized ones. Capsaicinoid has been shown to possess antioxidant activity with an ability to prevent the oxidation of human low-density lipoproteins.[12,13] However, application of capsaicinoid as a food antioxidant in fats and oils has not been explored except for the study of Yang et al., who found that capsaicin and tocopherol could prevent the thermal oxidation of pepper oil during frying.[13]

The present study was carried out (i) to test whether capsaicinoid was able to protect other vegetable oils from lipid oxidation, (ii) to compare its antioxidant potency with that of BHT, and (iii) to investigate its structure−antioxidant activity relationship.

MATERIALS AND METHODS

Materials. Canola oil without addition of any synthetic antioxidants was obtained from Lam Soon Marketing Service Ltd. (Kowloon, Hong Kong). Although no synthetic antioxidants were added, other natural antioxidants such as α-tocopherol are most likely present in canola oil. Capsaicinoid with a purity of 95% was obtained from Henan Bis-Biotech Co., Ltd. (Henan, China).

HPLC Analysis. To check the purity and composition, the individual capsaicinoid derivatives were separated and analyzed in a HPLC system having a C-18 column (250 mm × 4.6 mm, 5 μm) at 25 °C and eluted with a mixture of methanol and water at a flow rate of 1 mL/min. A UV detector was used to quantify each isomer at a wavelength of 280 nm. The initial mobile phase consisted of methanol and water in a ratio of 57:43, was programmed to 64:36 in 10 min, and was then held for an additional 25 min. HPLC analysis found that capsaicinoid consisted of 51.3% capsaicin, 36.2% dihydrocapsaicin, and 7.8% other capsaicinoids (Figure 1). Individual capsaicinoids were identified according to the retention times of authentic standards.

Oxygen Consumption Test. The oxygen uptake by canola oil was used as an oxidation marker as previously described.[14,15] In brief, 1 mL of hexane containing 200 mg of canola oil was placed in a glass tube (150 × 16 mm, o.d.) followed by the addition of 1 mL of ethanol containing various amounts of capsaicinoid or BHT. The oil and capsaicinoid were then mixed thoroughly. The solvents were removed under a gentle stream of nitrogen at 40 °C. The final concentrations of capsaicinoid were set at 50, 100, and 200 ppm, respectively, in canola oil, whereas that of BHT was set at only one dose of 200 ppm as a positive control. The reaction tubes (n = 6 for each concentration) were then flushed with air and sealed tightly with a rubber stopper obtained from an evacuated blood collection tube (100 × 16 mm, o.d., Becton-Dickinson, Rutherford, NJ, USA), which usually maintains a vacuum for 2−3 years. The sealed reaction tube was leak-free and was

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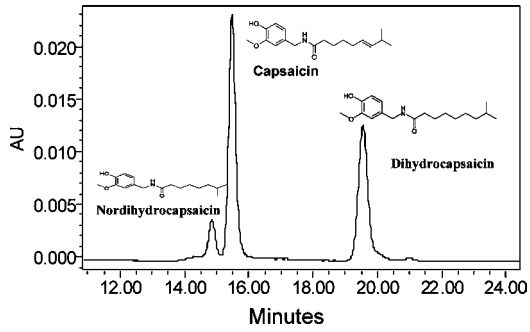

Figure 1. HPLC chromatogram and structures of three capsaicinoids.

verified by filling the tube with nitrogen gas and monitoring by gas chromatography (GC) for any decrease in headspace oxygen concentration. The reaction tubes were placed in a heating block, and the oxidation was conducted at three temperatures of 60, 90, 120, and 180 °C. The headspace oxygen was sampled periodically with a gastight syringe and analyzed in a Shimadzu GC-2010 gas–liquid chromatograph equipped with a $^1/_8$ in. × 6 ft stainless steel column packed with molecular sieve 5A (60/80 mesh) and a thermal conductivity detector. The percent oxygen in the headspace was calculated from the ratio of the oxygen to nitrogen.

Fatty Acid Analysis. An additional set of reaction tubes containing 200 mg of canola oil with various concentrations of capsaicinoid and 200 ppm BHT was similarly prepared and heated at 90 °C for the same period of time. At each designated time, the heated canola oil sample (20 mg) was taken for the fatty acid analysis. Fatty acids of heated canola oil were converted to their corresponding methyl esters in a mixture of 14% BF_3 in methanol (Sigma Chemical Co., St. Louis, MO, USA) and toluene (1:1, v/v) under nitrogen at 90 °C for 45 min.[14] Fatty acid methyl esters were analyzed on a HP Innowax capillary column (30 m × 0.5 μm, i.d. = 0.32 mm) in a Shimadzu GC-2010 gas–liquid chromatograph equipped with a flame ionization detector. Column temperature was programmed from 150 to 200 °C at a rate of 15 °C/min and then to 250 °C at a rate of 2 °C/min and was held for 15 min. Injector and detector temperatures were set at 250 and 270 °C, respectively. Helium was used as a carrier gas at a rate of 1.5 mL/min.

Derivatization of Capsaicinoid. Trimethylsilyl ether is a convenient way to derivatize a hydroxyl group on a compound.[16] To study the functional role of the hydroxyl group on capsaicinoids, the antioxidant activity of capsainoid–TMS derivative was determined and compared with that of its parent compound. In brief, capsaicinoid was converted to its TMS derivative using 200 μL of TMS reagent at 60 °C for 1 h. After evaporation of TMS reagent, the capsaicinoid–TMS derivative was added into canola oil at a concentration equivalent to 200 ppm BHT and then subjected to the oxygen consumption test and fatty acid analysis as described above at 90 °C.

Statistics. Data are expressed as the mean ± SD, n = 6 replicates. Data for the headspace oxygen consumption and fatty acid analysis were subjected to the two-way analysis of variance (ANOVA), and the means were compared between treatments by using Duncan's multiple-range test.

RESULTS

The antioxidant activity of capsaicinoid was examined in canola oil at four temperatures of 60, 90, 120, and 180 °C. As shown in Figure 2, the oxygen peak in the control canola oil became smaller after 72 h of heating at 60 °C, whereas that in canola oil containing 200 ppm capsaicinoid remained unchanged under the same experimental conditions, suggesting that capsaicinoid possessed the antioxidant activity. Compared with BHT at 200 ppm, capsaicinoid at the same concentration was more effective against lipid oxidation in canola oil heated at 60 °C (Figure 3). It was apparent that the inhibitory effect of capsaicinoid against lipid oxidation in heated canola oil was dose-dependent. Capsaicinoid was also effective against oxidation of canola oil at higher temperatures of 90, 120, and 180 °C (Figures 4–6). However, BHT at 200 ppm appeared to be more effective against the lipid oxidation than 200 ppm capsaicinoid at such high temperatures. Similar to that at 60 °C, the inhibiting effect of capsaicinoid on the oxidation of canola oil was dose-dependent (Figures 4–6).

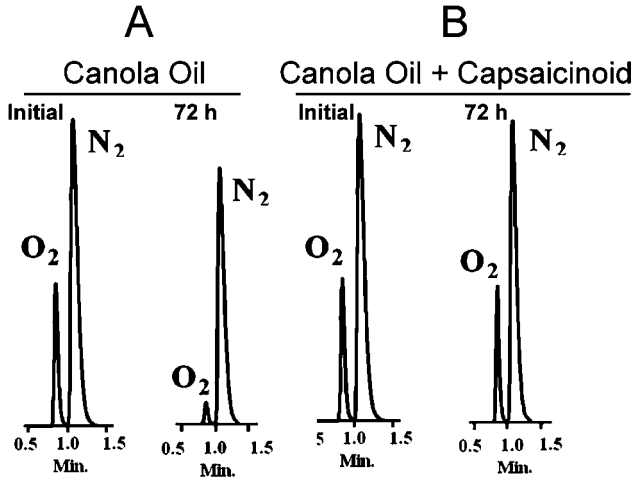

Figure 2. Gas chromatographic trace of headspace oxygen (O_2) and nitrogen (N_2) in reaction tubes containing canola oil (A) and canola oil + 200 ppm capsaicinoid heated at 60 °C for 72 h.

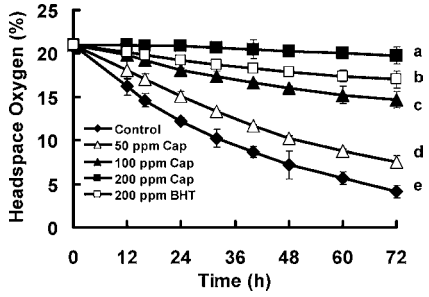

Figure 3. Headspace oxygen depletion trends in canola oil containing 50–200 ppm capsaicinoid (Cap) and 200 ppm butylated hydroxytoluene (BHT) heated at 60 °C. Data are expressed as the mean ± SD, n = 6 replicates. Curves with different letters (a–e) differed significantly at $p < 0.05$.

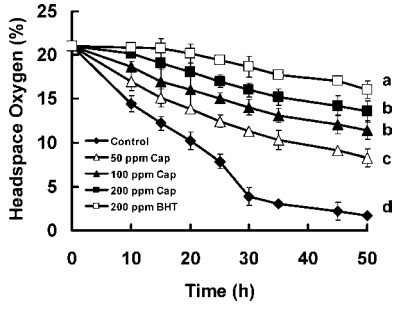

Figure 4. Headspace oxygen depletion trends in canola oil containing 50–200 ppm capsaicinoid (Cap) and 200 ppm butylated hydroxytoluene (BHT) heated at 90 °C. Data are expressed as the mean ± SD, n = 6 replicates. Curves with different letters (a–d) differed significantly at $p < 0.05$.

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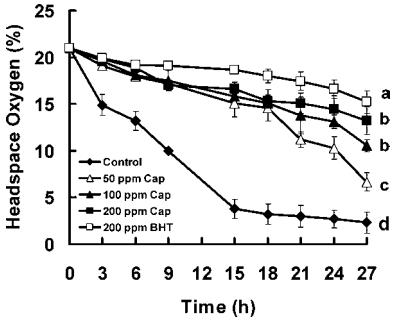

Figure 5. Headspace oxygen depletion trends in canola oil containing 50−200 ppm capsaicinoid (Cap) and 200 ppm butylated hydroxytoluene (BHT) heated at 120 °C. Data are expressed as the mean ± SD, n = 6 replicates. Curves with different letters (a−d) differed significantly at $p < 0.05$.

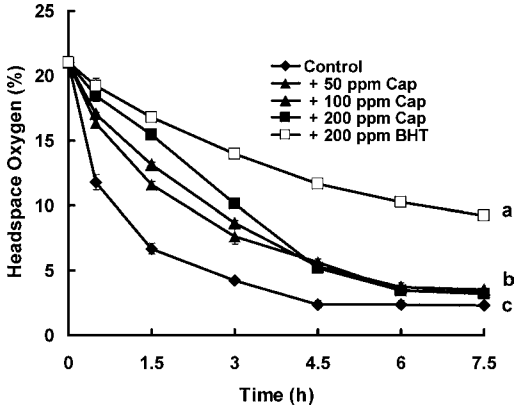

Figure 6. Headspace oxygen depletion trends in canola oil containing 50−200 ppm capsaicinoid (Cap) and 200 ppm butylated hydroxytoluene (BHT) heated at 180 °C. Data are expressed as the mean ± SD, n = 6 replicates. Curves with different letters (a−c) differed significantly at $p < 0.05$.

The oxidation of canola oil led to changes in its fatty acid profile. In general, oxidation of fats and oils leads to a proportional increase in saturated fatty acids and a proportional decrease in unsaturated fatty acids. As shown in Figure 7, two saturated fatty acids, namely, stearic acid and palmitic acid, were proportionally increased in the control canola oil, whereas addition of capsaicinoid suppressed such increases in these two fatty acids in a dose-dependent manner throughout the course of oxidation at 90 °C (Figure 7). In contrast, the three unsaturated fatty acids, namely, oleic acid, linoleic acid, and α-linolenic acid, in the control canola oil sample proportionally decreased throughout the course of 45 h of oxidation at 90 °C. Addition of 50, 100, and 200 ppm of capsaicinoid dose-dependently prevented such decreases in these three unsaturated fatty acids under the same experimental conditions (Figure 8).

The effect of capsaicinoid and its TMS derivative on the oxidation of canola oil was assessed at 90 °C (Figure 9). The results demonstrated that capsaicinoid was able to prevent the decrease in the headspace oxygen, whereas the capsaicinoid−TMS derivative lost the ability to prevent the oxidation of canola oil (Figure 9). The fatty acid analysis showed a similar trend, with capsaicinoid being able to prevent the decrease in linoleic and α-linolenic acid, whereas the capsaicinoid−TMS derivative lost such protection (Table 1).

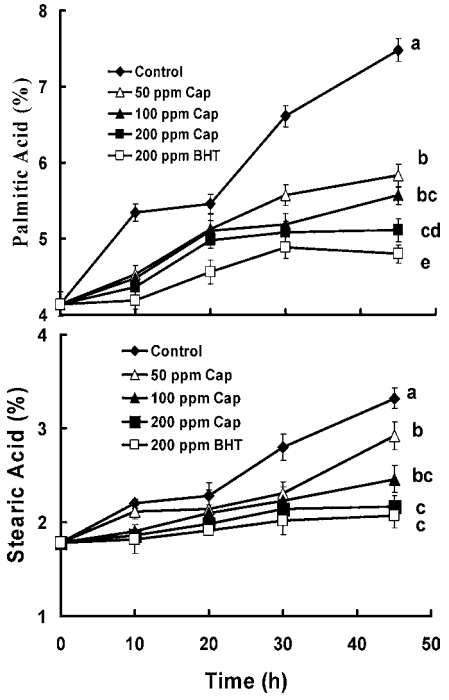

Figure 7. Effect of 200 ppm capsaicinoid (Cap) and 200 ppm butylated hydroxytoluene (BHT) on the content of stearic and palmitic acids of canola oil heated at 90 °C. Data are expressed as the mean ± SD, n = 6 replicates. Curves with different letters (a−e) differed significantly at $p < 0.05$.

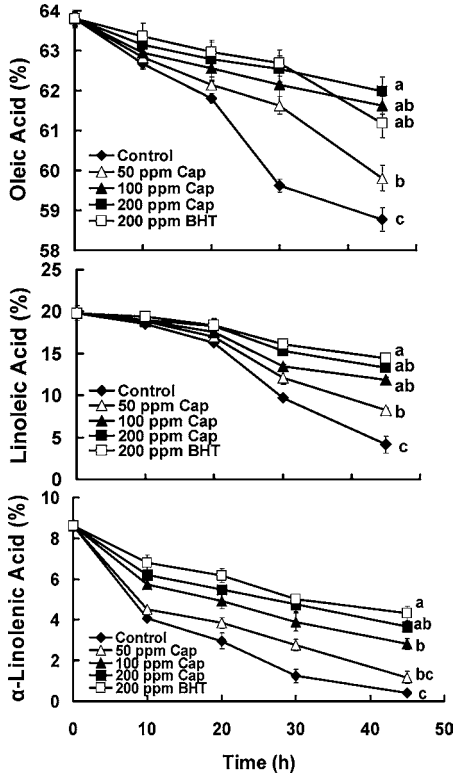

Figure 8. Effect of 200 ppm capsaicinoid (Cap) and 200 ppm butylated hydroxytoluene (BHT) on the content of oleic acid, linoleic acid, and α-linolenic acid in canola oil heated at 90 °C. Data are expressed as the mean ± SD, n = 6 replicates. Curves with different letters (a−c) differed significantly at $p < 0.05$.

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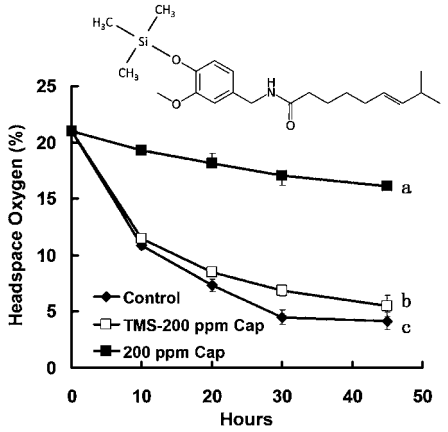

Figure 9. Headspace oxygen depletion trends in canola oil containing 200 ppm capsaicinoid (Cap) and equivalent 200 ppm trimethylsiloxy (TMS) capsaicinoid derivative heated at 90 °C. Data are expressed as the mean ± SD, $n = 6$ replicates. Curves with different letters (a–c) differed significantly at $p < 0.05$.

DISCUSSION

The present study was to investigate the feasibility of using capsaicinoid as an antioxidant in heated vegetable oil and to study the associated underlying mechanism. Both the headspace oxygen depletion and the fatty acid analysis clearly demonstrated that capsaicinoid was able to prevent lipid oxidation when the canola oil was heated at various temperatures. This result was in agreement with that of two previous studies showing that capsaicin was effective in inhibiting lipid oxidation in human low-density lipoproteins[12] and in pepper oil.[13] The present results affirmed that capsaicinoid was an effective antioxidant in vegetable oils, at least in canola oil. In comparson to BHT, the inhibitory effect of capsaicinoid was stronger at a low temperature of 60 °C and weaker at higher temperatures of 90, 120, and 180 °C. In general, a compound must have a dose-dependent activity if it is an antioxidant. The present study clearly demonstrated that capsaicinoid was effective against the lipid oxidation of canola oil in a dose-dependent manner regardless of the oxidation being monitored by the headspace oxygen depletion test or the fatty acid analysis.

The effectiveness of an antioxidant is governed by many factors including its activation energy, rate constant, oxidation–reduction potential, stability of its radical intermediate, thermal stability, and hydrophobicity.[17] Similar to BHT, which has only one hydroxyl group, capsaicinoid also possesses one hydroxyl moiety on the aromatic ring (Figure 1). Capsaicinoid was expected to possess an antioxidant activity similar to that of BHT for the following reasons. First, the hydroxyl group on an aromatic ring is more vulnerable to donation of a proton, with the resulting radical intermediate having a greater stability because of resonance delocalization.[18] Second, the long hydrocarbon side chain can make capsaicinoid more hydrophobic and soluble in canola oil, therefore rendering it a fat-soluble antioxidant. Third, its side chain also contains a polar amide (−NHCO−) group, which give capsaicin low volatility, leading to a persistent antioxidant activity during heating.

It was speculated that a hydroxyl group on the aromatic ring was accountable for the antioxidant activity of capsaicinoid observed in heated canola oil. To prove this structure–antioxidant activity relationship, the hydroxyl group on capsaicinoid was derivatized to its corresponding trimethylsiloxy group [$-O-Si(CH_3)_3$]. The present data clearly demonstrated that the blockage of the hydroxyl group led capsaicinoid to lose its antioxidant activity (Figure 9 and Table 1). The data proved that the hydroxyl moiety was indeed the functional group which rendered capsaicinoid an antioxidant.

In summary, we examined the antioxidant property of capsaicinoid and found its hydroxyl group was accountable for its antioxidant activity. Capsaicinoid was an effective antioxidant, which was more effective at a low temperature but less effective at a high temperature, compared with BHT, in preventing the lipid oxidation of canola oil. Capsaicinoid exhibited a dose-dependent antioxidant activity and could be further explored as a natural antioxidant in processed foods. On the one hand, application of capsaicinoid as an antioxidant may be limited only to spicy foods due to its burning sensation and pungency. On the other hand, capsaicinoid has advantages over synthetic antioxidants because in addition to its role as an antioxidant, capsaicinoid exhibits some additional health benefits associated with energy expenditure, the cardiovascular system, and reduction in plasma cholesterol.

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Notes

The authors declare no competing financial interest.

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Table 1. Effect of 200 ppm Capsaicinoid (Cap) and Equivalent 200 ppm Trimethylsiloxy (TMS) Capsaicinoid Derivative on the Fatty Acid Composition of Canola Oil (Weight Percent of Total Fatty Acids) Heated at 90°C[a]

	palmitic	stearic	oleic	linoleic	α-linolenic
unheated canola oil	4.14 ± 0.16	1.78 ± 0.04	63.80 ± 0.16	19.81 ± 0.29	8.62 ± 0.22
10 h at 90 °C					
heated canola oil	5.28 ± 0.15 a	2.31 ± 0.11 a	62.66 ± 0.14 b	17.77 ± 0.73 b	4.05 ± 0.27 b
+ 200 ppm Cap	4.40 ± 0.32 b	1.82 ± 0.12 b	63.28 ± 0.15 a	18.92 ± 0.23 a	6.21 ± 0.41 a
+ 200 ppm TMS Cap	5.09 ± 0.14 a	2.27 ± 0.13 a	62.90 ± 0.17 b	17.86 ± 0.32 b	4.79 ± 0.11 b
45 h at 90 °C					
heated canola oil	7.55 ± 0.35 a	3.40 ± 0.14 a	61.10 ± 0.15 a	4.05 ± 0.13 b	0.40 ± 0.21 b
+ 200 ppm Cap	5.28 ± 0.16 b	2.22 ± 0.18 b	61.87 ± 0.14 a	13.46 ± 0.70 a	3.65 ± 0.17 a
+ 200 ppm TMS Cap	7.01 ± 0.33 a	3.16 ± 0.12 a	61.39 ± 0.22 a	5.24 ± 0.91 b	0.44 ± 0.22 b

[a]Data are expressed as the mean ± SD, $n = 6$. Means with different letters in the same column and the same time point differ significantly, $p < 0.05$.

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