Butylated Hydroxytoluene in Edible Vegetable Oils from Local Markets of Chiang Mai and Mae Hong Son and Its Thermal Stability in Different Cooking Conditions

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ABSTRACT

Butylated hydroxytoluene (BHT) was widely used to protect oxidation and rancidity of food products containing fats and oils since it had high antioxidant activity. In this study, samples of edible vegetable oils from 25 local markets in Chiang Mai province and Mae Hong Son province were determined for BHT contents by gas chromatography-mass spectrometry (GC-MS). Of 18 vegetable oil samples, 3 palm oil samples from the local markets contained BHT in the range of 6.8-86.1 µg/g. The extracts of edible vegetable oil samples had 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity in the range of 7.5 to 75.8% which had gallic acid equivalent and BHT equivalent concentrations in the ranges of 6.5 to 65.9 mg/g and 17.2 to173.0 mg/g, respectively. Three different home-cooking conditions were tested for thermal stability of BHT in the samples of palm oil. It was found that cooking temperatures at 98-100 °C by boiling or stir-frying for 1-15 min lost BHT 24-31% in the oil, while deep frying at 200±10 °C for 5-15 min showed 46-62% loss of BHT.

Keywords: Butylated hydroxytoluene, edible vegetable oil, antioxidant activity

INTRODUCTION

Antioxidant compounds in food have an important role as a health protecting factor. They are supposed to protect living organisms from oxidative damage. Synthetic antioxidants are more easily available and have been used in a wide variety of oil products.[1] Although they are effective in protecting product quality in food, excess of these antioxidants added to food might produce toxicity or mutagenicities that may be harmful to human health.[2] The effects of BHT increase the susceptibility of mice to lung carcinogens and lung tumor development have broadened the concept of two-stage carcinogenesis and complement the evidence for initiation for other epithelial tissues in liver, colon, stomach, trachea, urinary bladder, and mammary glands.[3, 4] Many oil refiners and food processors continue to use butylated hydroxytoluene in vegetable oils because of its low cost.[5] The objectives of this study were (1) to determine BHT in edible vegetable oils including palm oil, soybean oil, corn oil, black sesame oil, rice bran oil, cotton seed oil and sunflower oil by GC-MS, (2) to assay antioxidant activity of these edible vegetable oils, and (3) to determine the thermal stability of BHT in different cooking conditions.

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MATERIALS AND METHODS

Materials

Standard solutions of 2,6-di-*tert*-butyl-4-methylphenol (BHT) and gallic acid were purchased from Sigma-Aldrich (Germany). Methanol and acetonitrile were purchased from JT Baker (USA). All chemicals were of analytical grade. Eighteen edible vegetable oil samples were purchased from local markets in Amphoe Sansai and Amphoe Muang, Chiang Mai, Thailand.

Preparation of BHT and gallic acid standards

A standard stock solution 0.1% (w/v) of BHT and gallic acid were dissolved in acetonitrile. The volumetric flask was shaken until a homogenous and clear solution was formed. The stock solution was covered with aluminum foil and stored in a refrigerator (~4 °C). Fresh standard solutions were prepared by dilution of an appropriate amount of the stock solution in acetonitrile on the day of use.

Sample preparation

One gram of each edible vegetable oil sample was put into a 20 ml sample bottle and then 2 ml of acetonitrile were added, secured the screw, shook and sonicated for 15 min. The sample extract was kept in a freezer at -20 °C for 1 hour. The clear portion was collected and sonicated. 1-2 µl of the sample solution was injected to a GC-MS system.

Measurement of radical scavenging activity

The method was adapted from Erasto *et al*[6] and Miraliakbari and Shahidi.[7] Briefly, 2 ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution (0.1 mM) in methanol was mixed with 1 ml of oil extracts (ten-fold dilution) and mixed thoroughly, left to stand for 30 min in the dark until stable absorption values were obtained. BHT and gallic acid were used as reference antioxidant compounds. The absorbance of remaining DPPH radicals was read at 517 nm using a spectrophotometer (HITACHI, Model U-1100, Japan). Analysis of each assay solution was done in triplicate. The scavenging of DPPH radicals was calculated according to the following equation:

DPPH radical scavenging activity (%) =

$$[(A_{control} - A_{sample})/A_{control}] \times 100$$

Thermal stability of BHT in palm oil

To investigate thermal stability of BHT in palm oil samples, the oil was heated in an electric pan, with temperature controlled at 200±10 °C. This condition was similar to a deep fried cooking condition in home kitchen of Thai people. At each 5 min interval, an aliquot of the fried oil (2 ml) was collected for extraction by acetonitrile and subjected to GC-MS analysis. Cooking condition by heating oil in boiling water with temperature controlled at 98 °C, at 5 minute interval, an aliquot of the cooking oil (2 ml) was collected for extraction and analysis. Cooking condition by stir-frying, the oil was stir-fried in mixture of water:oil (1:10), temperature was controlled at 100±5 °C. At 1 minute interval, an aliquot of stir-fried oil (2 ml) was collected for extraction and analysis. Preparation of these samples was described in the above preparation of samples. 1 µl of the sample extract solution was injected to a GC-MS system.

GC-MS analysis

GC-MS analysis was performed using Agilent 6890 (Agilent Technologies, USA) equipped with column HP-5MS (Agilent Technologies), 30 m x 250 µm i.d., 0.25 µm film thickness and Agilent MSD 5973 (Agilent Technologies) quadrupole mass spectrometer with an electron impact (EI) ion source as a detector. Carrier gas was Ultra high purity (UHP) helium at 1.0 ml/minute. Oven

temperature was: initial 40 °C, held for 3 minutes, programmed to 300 °C at 30 °C/minute. The detector was operated at 70 eV with full scan (30~550 amu). Ion source temperature was 230 °C. The temperature of quadrupole was 150 °C. Software used was NIST98 Library and WILEY Library mass database. Samples were injected in splitless mode.

RESULTS AND DISCUSSION

The BHT standard was determined based on the sample solution. The retention time of BHT standard was 8.84 minutes (Figure 1).

In the quantitation results of BHT of eighteen edible vegetable oil samples, three samples were found to contain BHT in the range of 6.8-86.1 mg/g (Table 1). The difference of BHT content in each sample depends on the manufacturer of the palm oil. Palm oil from the same manufacturer, but collected from different markets, showed almost the same BHT values.

The result showed that the 18 edible vegetable oil samples had DPPH radical scavenging activity in the range of 7.5-75.8%, 6.5-65.9 mg/g of gallic acid equivalent and 17.2-173.0 mg/g of BHT equivalent. Palm oil (1) – (7) had activity ranged from 40.2% to 75.8%, gallic acid equivalent and BHT equivalent in the ranges of 35.0-65.9 mg/g and 91.7-173.0 mg/g, respectively. Palm oil (3) has the highest activity than other palm oil and other vegetable oils (Table 2). All samples of edible vegetable oil showed antioxidant activity, but BHT were not detected in some samples, because of different antioxidants (natural or synthetic) used in various products.

Table 2 shows that the 18 edible vegetable oil samples have DPPH radical scavenging activity in the range of 7.5-75.8%, 6.5-65.9 mg/g of gallic acid equivalent and 17.2-173.0 mg/g of BHT equivalent. Palm oil (1) – (7) had activity ranged from 40.2% to 75.8%, gallic acid equivalent and BHT equivalent in the ranges of 35.0-65.9 mg/g and 91.7-173.0 mg/g, respectively. Palm oil (3) has the highest activity than other palm oil and other vegetable oils. All samples of edible vegetable oil showed antioxidant activity, but BHT were not detected in some samples, because of different

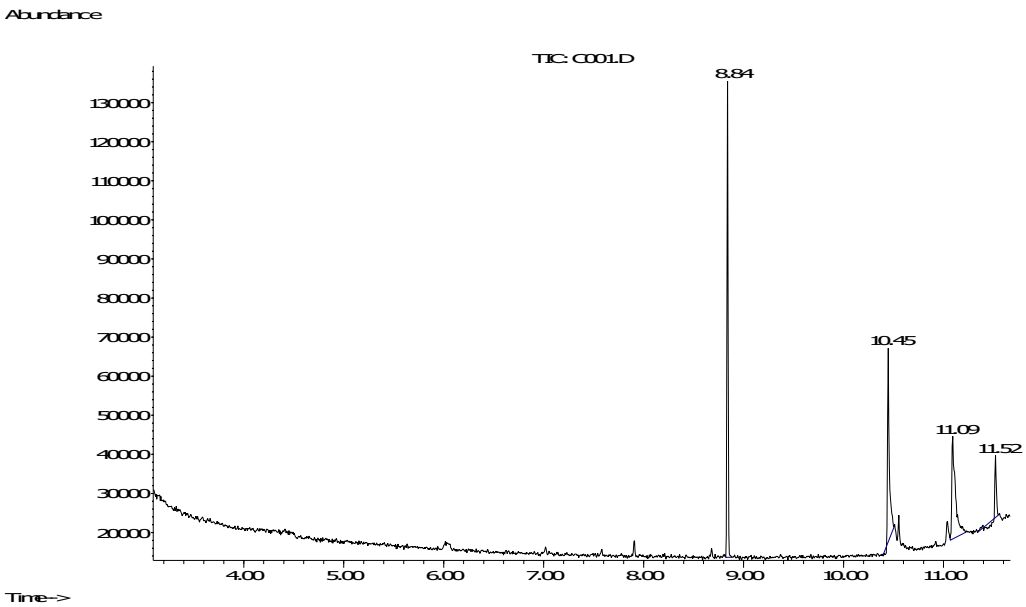

Figure 1 GC chromatogram of BHT detected from a palm oil extract.

Table 1 Content of BHT detected in the palm oil samples by GC-MS

Palm oil	BHT (µg/g)			
Sample #	1	2	3	Mean ± SD
(1)	85.9	86.1	86.2	86.1 ± 0.2
(2)	18.0	18.0	18.1	18.0 ± 0.1
(3)	6.8	6.7	6.8	6.8 ± 0.1

Note: BHT was not detected in other oil samples including 4 palm oil samples, 4 soybean, 2 corn oil, 2 black sesame oil, 1 rice bran oil, 1 cotton seed oil and 1 sunflower seed oil sample(s).

antioxidants (natural or synthetic) used in various products.

Cooking such as deep frying, boiling in water and stir-frying methods could affect BHT content in the oil. This study demonstrated that deep frying was the best method to decrease BHT from 100% to 46% at 5 min and 62% at 15 min. Heating of oil in boiling water and stir-frying could decrease the BHT to about 24-31% within 1-15 min. In other study, temperature can drastically affect the commercial antioxidants. BHT is effective as antioxidants at temperatures up to 175 °C and exhibiting only 25 to 30% inactivation.[8]

In conclusion, BHT in oil samples was generally safe for consumption and we found only one sample contained BHT over the limit. The maximum level of BHT limited by Thai Food Regulations (Ministry of Public Health, Thailand) is 75 mg/kg of BHT for edible fats and oils. This method should be applied for effective analysis of BHT antioxidant in other vegetable oils, edible fats and toxic residues in foods. One of the important applications of GC-MS was for identifying compounds using both the retention times and the relative abundances of the characteristic fragment ions, thus increasing accuracy and providing reliable results. Cooking temperatures and time as well as cooking method affected BHT contents in foods.

Table 2 DPPH radical scavenging activity of edible vegetable oils (mean ± SD)

Samples	DPPH radical scavenging activity (%)	Gallic acid equivalent (mg/g)	BHT equivalent (mg/g)
Palm oil (1)	48.9±0.1	42.5±0.1	111.7±0.3
Palm oil (2)	43.1±0.1	37.5±0.0	98.3±0.0
Palm oil (3)	75.8±0.1	65.9±0.1	173.0±0.3
Palm oil (4)	43.2±0.1	37.6±0.1	98.5±0.2
Palm oil (5)	40.2±0.1	35.0±0.1	91.7±0.2
Palm oil (6)	45.1±0.1	39.2±0.2	102.9±0.4
Palm oil (7)	45.5±0.1	39.6±0.1	103.8±0.2
Soybean oil (1)	14.9±0.0	13.0±0.1	33.8±0.1
Soybean oil (2)	18.5±0.0	16.1±0.1	42.3±0.1
Soybean oil (3)	19.3±0.1	16.8±0.2	44.0±0.4
Soybean oil (4)	13.6±0.2	11.8±0.2	31.1±0.7
Corn oil (1)	7.5±0.0	6.5±0.1	17.2±0.1
Corn oil (2)	14.8±0.0	12.9±0.1	33.7±0.1
Black sesame oil (1)	20.7±0.1	18.0±0.1	47.1±0.3
Black sesame oil (2)	25.2±0.1	21.9±0.1	57.5±0.2
Rice bran oil (1)	30.3±0.1	26.4±0.2	69.1±0.5
Cotton seed oil (1)	7.8±0.1	6.8±0.2	17.8±0.4
Sunflower seed oil (1)	9.7±0.2	8.4±0.3	22.1±0.7

Table 3 Stability of BHT in 3 different cooking conditions

Cooking condition	BHT (%)				Loss of BHT (%)			
	0 min	1 min	5 min	15 min	0 min	1 min	5 min	15 min
Deep fried	100	-	54	38	0	-	46	62
Boiled	100	-	73	69	0	-	27	31
Stir-fried	100	76	71	-	0	24	29	-

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บิวทิเลตเตตไฮดรอกซีโทลูอีน ในน้ำมันพืชสำหรับบริโภคที่มีจำหน่ายในตลาดของเชียงใหม่และแม่ฮ่องสอน และความคงตัวต่อความร้อนในสภาวะการปรุงอาหารที่แตกต่างกัน

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บทคัดย่อ

บิวทิเลตเตต ไฮดรอกซีโทลูอีน (บีเอชที) ใช้อย่างกว้างขวางสำหรับป้องกันการออกซิเดชันของผลิตภัณฑ์อาหารซึ่งทำจากไขมันและน้ำมันเนื่องจากมีฤทธิ์ต้านอนุมูลอิสระสูง ในการศึกษานี้ได้วิเคราะห์หาปริมาณของบีเอชทีในน้ำมันสำหรับบริโภค ซึ่งมีจำหน่ายในตลาดท้องถิ่น 25 แห่งของจังหวัดเชียงใหม่และจังหวัดแม่ฮ่องสอน ด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี พบว่าน้ำมันปาล์มจำนวน 3 ตัวอย่างมีบีเอชที 6.8-86.1 ไมโครกรัม/กรัม การตรวจฤทธิ์ต้านอนุมูลอิสระด้วยสารละลาย 1,1-ไดฟีนิล-2-พิคริลไฮดราซิล (ดีพีพีเอช) พบฤทธิ์การต้านอนุมูลอิสระ 7.5 ถึง 75.8% เมื่อเทียบกับสารมาตรฐานกรดแกลลิกและสารมาตรฐานบีเอชทีเทียบเท่าระดับ 6.5 ถึง 65.9 ไมโครกรัม/กรัมของกรดแกลลิกและ 17.2 ถึง 172.7 ไมโครกรัม/กรัมของบีเอชทีตามลำดับ การทดสอบผลของวิธีการหุงต้มต่อความคงตัวของบีเอชทีในตัวอย่างน้ำมันปาล์ม 3 วิธีการ พบว่าอุณหภูมิการหุงต้มอาหาร 98-100 ^{o}ซ เป็นเวลา 1-15 นาที สามารถลดความเข้มข้นของบีเอชทีในน้ำมันได้ร้อยละ 24-31 โดยวิธีการต้มหรือผัดในน้ำมัน ขณะที่การทอดในน้ำมันที่อุณหภูมิสูง 200±10 ^{o}ซ เป็นเวลา 5-15 นาที สามารถลดบีเอชทีในน้ำมันได้มากถึงร้อยละ 46-62

คำสำคัญ: บีเอชที, น้ำมันพืชสำหรับบริโภค, ฤทธิ์ต้านอนุมูลอิสระ