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Effects of Extraction Solvent on Gas Chromatographic Quantitation of BHT and BHA in Chewing Gum

HSIU-JUNG LIN¹, MEI-LIN WANG², YOUK-MENG CHOONG^{1*}, CHUNG-WEN CHEN³,
BAO-SHYUNG HWANG¹, SHYR-LIN TSAI¹, LUNG-CHI CHUNG¹ AND MIN-HUA YANG³

¹ Department of Food Science and Technology, TaJen Institute of Technology
20, Wei-shin Rd., Yan-puu Hsiang, Pintung Hsien 907, Taiwan, R.O.C.

² Department of Food Health, Chia-Nan University of Pharmacy and Science
60, Al-jen Rd., Sec. 1, Pau-an, Jen-teh Hsiang, Tainan Hsien 717, Taiwan, R.O.C.

³ Department of Food and Nutrition, Hung-Kuang University
34, Chung-Chi Rd., Sha-lu, Taichung Hsien 407, Taiwan, R.O.C.

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ABSTRACT

In this study, a simple and rapid method was developed for the determination of BHT (2,6-di-*t*-butyl-*p*-hydroxytoluene) and BHA (*t*-butyl-4-hydroxyanisole) in chewing gum using solvent extraction combined with gas chromatography. The results showed that among seven types of extraction solvent, diethyl ether was the best, as chewing gum dissolved and dispersed in diethyl ether, gave the highest yield of BHT and BHA from chewing gum. Recovery studies were performed on chewing gum, spiked with both BHT and BHA at 100~200 µg/g each. The recoveries of both BHT and BHA were 99~101% (CV: 1.5~3.2%) and 94~99% (CV: 7.1~8.6), respectively. The coefficients of variation were less than 8.6%. Fifteen chewing gum samples were analyzed by the current method. Both the BHT and BHA contents were found to be 0~296 µg/g and 0~133 µg/g, respectively. After chewing for 15 min, 70% of the total soluble compounds and 50% of BHT and BHA were released from chewing gum.

Key words: chewing gum, artificial antioxidants, solvent extraction, quantitation, gas chromatography.

INTRODUCTION

Rancidification due to oxidation, the most prominent problem of oil and fat-containing foods, not only influences food quality, but also risks human health. It's a very important task to prevent and reduce oxidation of fat. The addition of antioxidants is one of the major methods currently used to prevent oxidation of fat⁽¹⁾.

Most of the currently used antioxidants are artificial antioxidants, e.g., BHT (2,6-di-*t*-butyl-*p*-hydroxytoluene) and BHA (*t*-butyl-4-hydroxyanisole). Due to their good stability, low price, and easy to obtain, artificial antioxidants are very popular. But their safety is suspicious to a lot of consumers because the potential risk factors have been discovered⁽²⁻⁶⁾. From the food sanitary point of view, people can predict the usage of artificial antioxidants will be strictly restricted.

In accordance with the management of food sanitary and to improve the quality of food manufacturing, it is essential to develop the analytical method for antioxidants in foods. Current official antioxidants analysis methods available in Taiwan are only for BHA and BHT⁽⁷⁾. Due to synergistic effect of combination usage of antioxidants⁽⁸⁾, the addition of two or more antioxidants in foods are popular in Taiwan. It is very urgent to develop the analyti-

cal methods, which can simultaneously detect multiple antioxidants in foods.

The analytical methods of antioxidants in foods are colorimetric method⁽⁹⁾, UV spectrophotometric method⁽¹⁰⁻¹²⁾, paper and thin-layer chromatographic methods⁽¹³⁻¹⁴⁾, gas chromatographic method⁽¹⁵⁻²⁰⁾, and high performance liquid chromatographic method⁽²¹⁻²⁶⁾. The application of abovementioned methods is limited, due to the complicate operation procedures and unable to detect multiple target antioxidants at the same time.

There are lots of gas chromatography methods developed for the determination of antioxidants in foods⁽¹⁵⁻²⁰⁾. In these methods, BHA or BHT is extracted by solvent extraction or steam fractionation, and then BHA/BHT or its derivatives are injected into a GC analyzer for analysis⁽¹⁵⁾. The pretreatment procedures are complicated, and the conditions in formation of derivatives would interfere the reproducibility and quantitative accuracy in the analysis of antioxidants.

Chewing gum is manufactured by mixing of the chewing gum base with sweetening agent, seasoning, emulsifier, wax, antioxidants (usually BHT and/or BHA), and other ingredients. Currently, there is no accurate analytical method available for quantification of antioxidants. When natural or artificial gum being used as the chewing gum base, and acetonitrile in the official edible oil antioxidant determination method⁽⁷⁾ is applied for the extraction of

* Author for correspondence. Tel:886-8-7624002 ext 352;
Fax:886-8-7621972; E-mail:ymchoong@ccsun.tajen.edu.tw

antioxidants in chewing gum, the chewing gum will aggregate and can't be dissolved or dispersed in acetonitrile solution, and this prohibits the accurate quantitation of antioxidants in chewing gum. In this study, we plan to compare the solubility, dispersion, and extraction efficiency among 7 different organic solvents, including methanol, isopropanol, acetonitrile, acetone, n-hexane, diethyl ether, and ethyl acetate, when applied in analyzing antioxidants in commercial chewing gums. The solvent extract is injected directly into a GC analyzer for the determination of antioxidants. A simple analytical method for antioxidants, BHA, and BHT, in chewing gums will be established. In this study, determination of the antioxidant contents in commercial chewing gums and the change of residue antioxidants in gums after chewing are also conducted to validate the quantitation method we developed.

MATERIALS AND METHODS

I. Materials

There were 15 commercial chewing gums purchased from supermarkets in Pintung and Tainan areas. Standard solutions, n-decanol, n-dodecanol, n-tetradecanol, and hexadecanol, all in purity > 99%, were from TCI (Tokyo, Japan). LC grade methanol, isopropanol, acetonitrile, acetone, n-hexane, diethyl ether and ethyl acetate were purchased from ALPS Chemical Co, Taiwan.

II. Methods

(I) Preparation of standard solutions

1. Preparation of 0.01% Standard Solutions

Individually weighted 0.1 g (in precision to 0.0001g) of n-tetradecanol (14OH), BHT and BHA, was dissolved in diethyl ether and volumetrically adjusted to make 100 mL.

2. Preparation of 0.05% Standard Solutions

0.5 g (in precision to 0.0001 g) of 14OH was weighted, dissolved in diethyl ether, and volumetrically adjusted to make 100 mL.

(II) Determination of the relative response factor (RRF) of BHT and BHA against n-tetradecanol

0.1% (w/v) 14OH was mixed with 0.1% (w/v) BHT and BHA in different ratios (BHT, BHA: 14OH = 1:5, 1:2, 1:1, 2:1 and 5:1, v/v), and then the ratios of the GC area under curve (AUC, in triplicate) and the concentration ratios of BHT and BHA against internal standard, 14OH, were calculated. The relative response factors (RRFs) of BHT and BHA against 14OH were calculated according to equation (1).

$$RRF = (A_{BHA \text{ or } BHT}) / (W_{BHA \text{ or } BHT}) \div (A_{IS}) / (W_{IS}) \dots\dots\dots(1)$$

When a known concentration of internal standard, 14OH, was added, the quantity of BHT and BHA in chewing gum samples could be calculated according to equation (2) and (3), respectively.

$$BHT (\mu\text{g/g}) = (A_{BHT}) / (A_{IS}) \times (W_{IS}) / (RRF_{BHT}) \times (1/W) \dots(2)$$

$$BHA (\mu\text{g/g}) = (A_{BHA}) / (A_{IS}) \times (W_{IS}) / (RRF_{BHA}) \times (1/W) \dots(3)$$

$A_{BHT \text{ or } BHA}$: GC AUC of BHT and BHA;
 $W_{BHT \text{ or } BHA}$: weight (μg) of BHT and BHA;
 A_{IS} : GC AUC of internal standard, 14OH;
 W_{IS} : weight (μg) of internal standard, 14OH;
 W : sample weight (g).

(III) Study of the extraction conditions for BHT and BHA

1. Dissolution or Dispersion of Chewing Gum in Solvents

One gram of chewing gum sample was added into a test tube with cap (15 mm \times 20 cm), then 8 mL of test solvent, including methanol, ethanol, isopropanol, acetonitrile, acetone, ethyl acetate, diethyl ether, and n-hexane, was added. After sonicating by Ultrasonic LC (Elma, Germany) for 5 min, the dissolution or dispersion of the sample in the solvent will be monitored.

2. The Extraction Efficiency of BHT and BHA from Solvents

One gram of chewing gum sample was added into a sample vial (20 mL), then 8 mL of test solvent, including methanol, ethanol, isopropanol, acetonitrile, acetone, ethyl acetate, diethyl ether, and n-hexane, and 200 μL of 0.5% internal standard solution, 14OH, was added. Secured the screw and sonicated for 5 min, 0.1 μL of the sample solution was injected into a GC for analysis. Blank sample, without chewing gum, was also analyzed according to the same procedure as abovementioned.

3. Quantitation of BHT and BHA in Chewing Gum Samples

Chewing gum sample was cut into small pieces, and 0.1 g of the sample was added into a 20-mL vial with cap. After the addition of 2, 3, 4, 5, 8, or 10 mL of diethyl ether and 0.2 mL of 0.5% (w/v) internal standard solution, the vial was sealed and sonicated for 5 min to make solid sample disperse thoroughly. The procedure was repeated for 1 to 5 times. 0.1 μL of the supernatant solution was injected directly into a GC for analysis. This was the procedure how the quantitation of BHT and BHA was followed.

(IV) Recovery study

0.1 and 0.2 mL of 0.1% (w/v) BHT and BHA standard

solution (equivalent to 100~200 μg) was added respectively into 1.0 g of chewing gum sample, and then 0.2 mL of 0.5% (w/v) 14OH in ethyl acetate solution and 5 mL diethyl ether were added. After sonicating for 5 min, 0.1 μL of sample solution in diethyl ether layer was injected directly into a GC for analysis. Each sample was measured in triplicate to determine the recovery of BHT and BHA.

(V) Quantitation of BHT and/or BHA in chewing gum samples after chewing

Chewing gum sample (about 3.2 g), after chewing for 0, 5, 15, 30 and 60 min, was weighted and its water content was measured. The chewing process was done by two different persons and each sample was analyzed in duplicate. The quantities of BHT and BHA were measured, and the mean values of four measures were determined.

(VI) Conditions of gas chromatography

A GL Science Model G-390B gas chromatography (Tokyo, Japan) equipped with a flame ionization detector (FID, H_2 and air flow at 30 and 300 mL/min, respectively) was used in this study. Separation column was ChromPack (The Netherlands) CP-SIL 24 CB megapore capillary column (0.53 mm \times 30 m, 1.5 μm). The operational temperature was set as followed: injection site, 240°C; detector temperature, 290°C; and initiation temperature of oven at 100°C for 2 min. The temperature was accelerated to 230°C, in the rate of 10°C/min, and kept for 1 min. The temperature was then accelerated to 300°C in the rate of 40°C/min. The carrier gas used was nitrogen, N_2 , in a flow rate of 4 mL/min. The injection volume was set at 0.1 μL , and the splitless injection mode was chosen.

RESULTS AND DISCUSSIONS

I. Evaluation of the GC Conditions

There were a lot of publications concerning the detection of antioxidants in edible oil using GC⁽¹⁵⁻²⁰⁾, but accurate method for quantitation of antioxidants in chewing gums was not available. Because chewing gum base came from natural or artificial gum, when acetonitrile was used to extract antioxidants in chewing gum samples, according to official method⁽⁷⁾ the chewing gum samples were aggregated and were not dissolved or dispersed in acetonitrile. Hence, the antioxidants could not be effectively extracted from the chewing gum samples. In this study, we evaluated the solubility, dispersion capability, and extraction effectiveness of antioxidants from chewing gum samples with several organic solvents, including methanol, isopropanol, acetonitrile, acetone, hexane, diethyl ether, and ethyl acetate. The extract from organic solvent was injected directly into a GC for quantitation of antioxidants, BHT and BHA, in chewing gums.

In the selection of separation column, polar CP Wax

58 CB (30 m \times 0.53 mm), mid-polar CP SIL 24 CB (30 m \times 0.53 mm), and non-polar CP SIL 8 CB and CP SIL 5 CB (30 m \times 0.53 mm) were compared. The results indicated, in analyzing BHT and BHA, mid-polar CP SIL 24 CB (30 m \times 0.53 mm) was the most appropriate column. The splitless injection mode was chosen when organic extract was injected into a GC. The temperature accelerating procedures were followed as mentioned in Materials and Methods. The retention time of BHT and BHA was 8.95 and 9.67 min, respectively, as shown in Figure 1 and 2.

Small amount of n-decanol (10OH), n-dodecanol (12OH), n-tetradecanol (14OH), and n-hexadecanol (16OH) standard solution were sequentially added into BHT and BHA standard solutions. Sample solutions were analyzed according to the above mentioned GC condition. The internal standard solutions for analyzing BHT and BHA

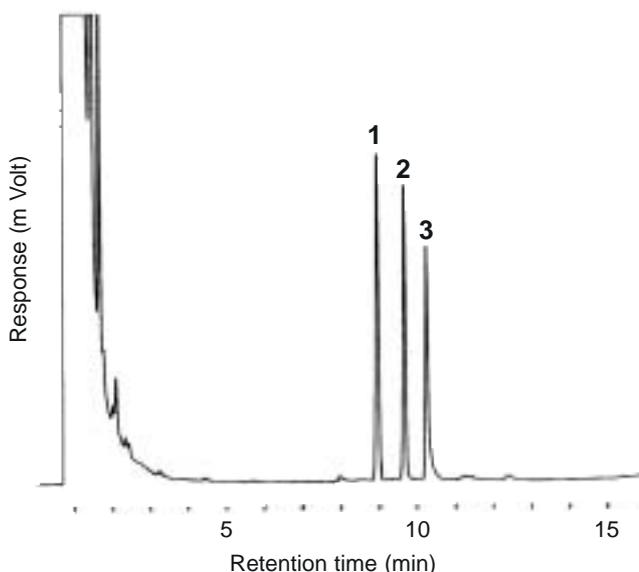


Figure 1. Gas chromatogram of BHT, BHA and internal standard, n-tetradecanol. Peaks: 1. BHT; 2. BHA; 3. 14OH (IS).

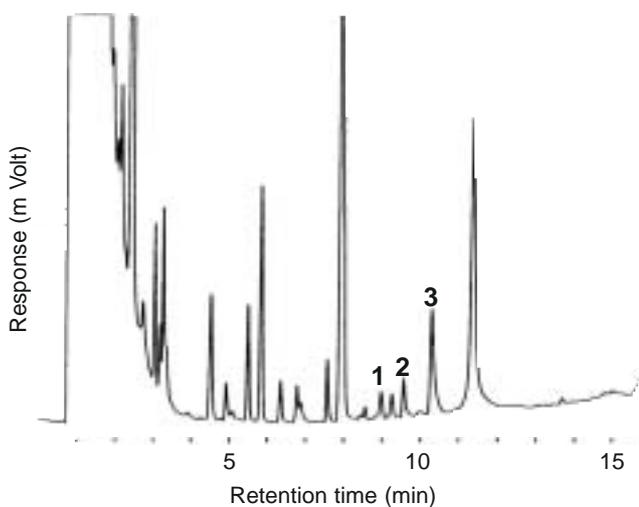


Figure 2. Gas chromatogram of antioxidants BHT and BHA in diethyl ether extracts of chewing gum. Peaks numbered same as in Fig. 1.

were determined based on the retention time of the sample solutions. The retention time of 10OH, 12OH, 14OH and 16OH standard solution were 6.23, 8.68, 10.28 and 11.76 min, respectively (data not shown). Among them, no overlapping of GC peaks between 14OH and organic extract of chewing gum sample was observed, which indicated 14OH would be appropriate as the internal standard (IS) in quantitation of BHT and BHA.

II. The RRF of BHT and BHA against Internal Standard

In this study, 14OH was chosen as the internal standard for quantitation of BHT and BHA in chewing gum samples. First of all, the RRF of BHT and BHA against internal standard was determined, and then the amount of BHT and BHA could be calculated according to equation (2) and (3). If we plot the AUC of BHT and BHA in GC against the individual concentration, each one of the regression coefficient (R^2) was higher than 0.99 (data not shown). The RRF of BHT and BHA against internal standard was 1.21 and 1.01, respectively, as shown in Table 1.

III. Extraction Conditions for BHT and BHA

(I) The Influence of solvents in solubility and dispersion on chewing gum samples

Chewing gum samples were added individually with methanol, ethanol, isopropanol, acetonitrile, acetone, ethyl acetate and n-hexane, and then observed after sonication.

Table 1. Relative response factor and retention time of BHT, BHA and n-tetradecanol, the internal standard, in gas chromatography

Compound	Relative response factor (RRF) ^b	Retention time (min) ^c
BHT	1.21	8.95
BHA	1.01	9.67
Tetradecanol, 14OH ^a	1.00	10.28

a: As the internal standard.

b: See "materials and method" for the determination of relative response factor.

c: CP-Sil 24 CB 0.53 mm × 30 m column was used. Oven temperature program = 100°C (2 min) → 10°C/min → 230°C (1 min) → 40°C/min → 300°C (1 min).

Table 2. Effect of extraction solvent on the quantitative determination of BHT and BHA in chewing gum by gas chromatography

Solvent ^a	Chewing gum status in solvent	Antioxidant content (μg/g) ^b	
		BHT	BHA
Methanol	Gummy	125.3 ± 15.4	38.9 ± 4.1
Ethanol	Gummy	131.9 ± 13.7	41.7 ± 3.9
Isopropanol	Gummy	140.4 ± 16.1	42.5 ± 5.4
Acetonitrile	Gummy	126.6 ± 13.4	46.3 ± 3.7
Acetone	Gummy	157.3 ± 9.7	43.5 ± 5.2
Ethyl acetate	Slightly dispersed	229.1 ± 15.1	79.8 ± 3.7
Diethyl ether	Dispersed powder	290.1 ± 13.2	125.5 ± 4.8
Hexane	Slightly dispersed	235.7 ± 14.8	93.6 ± 6.1

a: Solvent = 1 g chewing gum + 8 mL solvent.

b: Average of triplicate analyses.

Results were shown in Table 2. In polar solvents, methanol, ethanol, isopropanol, acetonitrile and acetone, chewing gum samples barely dissolved or dispersed; while in less polar solvents, ethyl acetate, diethyl ether, and n-hexane, chewing gum samples were partially or completely degraded and dispersed, especially in diethyl ether.

(II) Comparison of the extraction effectiveness of BHT and/or BHA by solvents in chewing gum samples

After addition of methanol, ethanol, isopropanol, acetonitrile, acetone, ethyl acetate and n-hexane, and through sonication procedure, 0.1 μL of chewing gum sample solutions in organic layer was injected directly into a GC for the analysis of BHT and BHA. The results were shown in Table 2. Because of poor dissolution or dispersion in polar solvents, methanol, ethanol, isopropanol, acetonitrile and acetone, less BHT and BHA, in the amount of 125~157 and 39~46 μg/g, respectively, were determined. In the other hand, due to chewing gum samples were partial or complete degradation and dispersion in less polar solvents, ethyl acetate, diethyl ether, and n-hexane, more BHT and BHA, in the amount of 230~290 and 90~126 μg/g, respectively, were determined. Among them, diethyl ether showed the best extraction effect, which BHT and BHA in the amount of 290 and 126 μg/g, respectively, were determined.

(III) The Influence of diethyl ether amount on the quantitation of BHT and BHA in chewing gum samples

After addition of 2, 3, 4, 5, 8, and 10 mL of diethyl ether, 1 g of chewing gum sample was used to determine the content of BHT and BHA. Results were shown in Figure 3. When 1 g of chewing gum sample was extract with 4~5 times volume of diethyl ether (4~5 mL), the maximum quantitation of BHT and BHA could be reached.

(IV) The influence of solvent extraction frequency on the quantitation of BHT and BHA in chewing gum samples

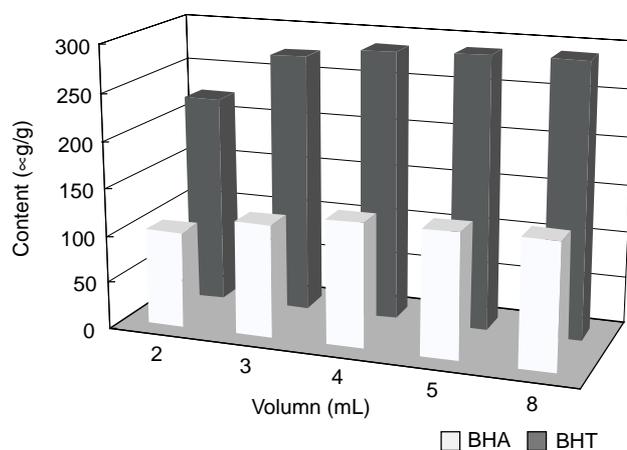


Figure 3. Effect of solvent volume of diethyl ether on the determination of BHT and BHA in chewing gum.

Table 3. Recoveries of the spiked BHT and BHA from one of the commercial chewing gum

Antioxidants	Blank ^a ($\mu\text{g/g}$) (A)	Spiked amount ($\mu\text{g/g}$) (B)	Amount determined ^b ($\mu\text{g/g}$) (C)	Recovery (%) ^c	CV (%) ^d
BHT	265.4 \pm 6.4	100.0	390.8 \pm 5.8	101.4	1.5
	265.4 \pm 6.4	200.0	469.7 \pm 15.8	99.0	3.2
BHA	131.9 \pm 3.4	100.0	225.7 \pm 19.3	93.8	8.6
	131.9 \pm 3.4	200.0	329.6 \pm 23.4	98.9	7.1

a: BHT and BHA content in 1.0 g of chewing gum.

b: Mean \pm S.D., n = 3.

c: Recovery (%) = (C - B) / A \times 100%.

d: Coefficient of variation were obtained from triplicate tests.

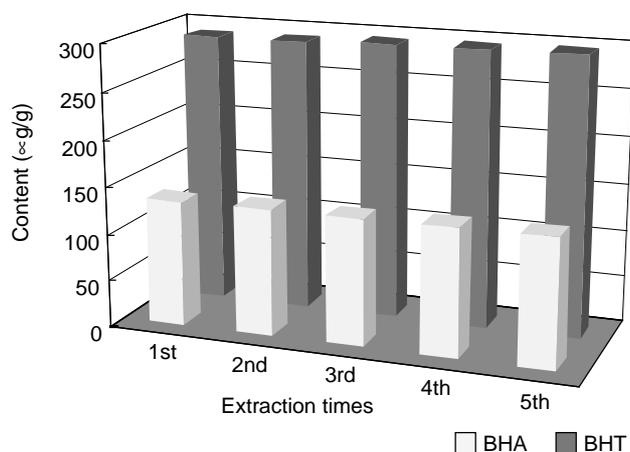


Figure 4. Effect of extraction times with diethyl ether on the determination of BHT and BHA in chewing gum.

In this study, we also evaluate the influence of extraction frequency (1~5 times) of diethyl ether on the quantitation of BHT and BHA in chewing gum samples. Results were shown in Figure 4. Only extraction by 5 mL of diethyl ether and a single 5 min sonication were needed to reach the maximum quantitation of BHT and BHA in chewing gum sample.

IV. Recovery Test

0.1~0.2 mL of 0.1%(w/v) BHT and BHA standards (equivalent to 100~200 μg) were individually added into 1.0 g of chewing gum samples, and then 0.2 mL of 0.5% (w/v) internal standard and 5 mL of diethyl ether were added. Results of GC analysis were shown in Table 3. The recovery rate of BHT and BHA were 99~101% and 94~99%, respectively, with coefficient of variance less than 8.6%. The results indicate the method we developed, with diethyl ether extraction in sonication and direct injection into a GC, is a simple, rapid and accurate method for quantitative analysis of BHT and BHA in chewing gum samples. Each sample takes only 15 min to complete the analysis. Gas chromatograms were shown in Figure 2. For quantitative analysis of BHT and BHA, the extraction reagent, diethyl ether, used in this study is more efficient and accurate compared with acetonitrile used in AOAC method⁽⁷⁾. This method is suggested as a routine method

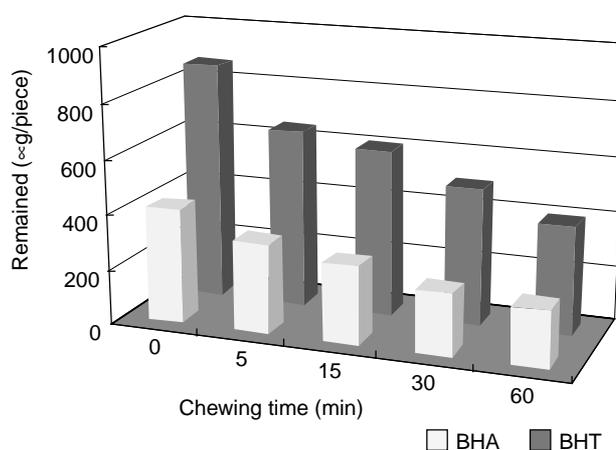


Figure 5. Changes of residual BHT and BHA remained in chewing gum during chewing.

for the quantitative analysis of BHT and BHA contents in foods like chewing gums.

V. The Influence of Chewing Time on the Extraction Effectiveness of BHT and BHA in Chewing Gum Samples

In this study, we also evaluate the changes of water content, soluble substance content, BHT and BHA contents in a piece of chewing gum, about 3.2 g, during chewing process. As shown in Figure 5 and 6, during chewing process, soluble substance contents, BHT and BHA contents in a piece of chewing gum reduced gradually. The water content, 5% before chewing, increased gradually with the induction of saliva, and reached maximum 25% after 15-min chewing. After 1 h chewing, the weight of chewing gum reduced to around 26% of its origin; while, the BHT and BHA contents reduced from original 869 $\mu\text{g}/\text{piece}$ and 417 $\mu\text{g}/\text{piece}$ to 396 $\mu\text{g}/\text{piece}$ and 204 $\mu\text{g}/\text{piece}$, respectively. That is to say, after 1 h chewing, almost 53% BHT and BHA was dissolved and ingested. Also shown in Figure 5 and 6, when soluble contents were extracted, the maximum amount (around 56%) of BHT and BHA in chewing gum sample was observed after 15 min chewing. More than 15 min chewing, the soluble contents and BHT and BHA were reduced dramatically. This indicates about 70% soluble contents were extracted after 15 min chewing; within which, about 56% BHT and BHA were extracted.

Table 4. BHT and BHA content of commercial chewing gum products

Sample	Weight/piece (g/piece)	Antioxidants content ($\mu\text{g/g}$) ^b		BHT + BHA ($\mu\text{g/g}$) ^b	Total antioxidant Contents ($\mu\text{g/piece}$) ^c
		BHT	BHA		
A (Wrig-1)	3.21	277.6 \pm 12.4	131.7 \pm 8.4	409.3	1313.9
B (Wrig-2)	3.21	281.4 \pm 15.3	124.6 \pm 9.5	406.0	1303.3
C (Wrig-3)	3.21	287.2 \pm 9.2	119.6 \pm 7.3	406.8	1305.8
D (Ext-1)	2.76	233.8 \pm 8.7	ND	233.8	645.3
E (Ext-2)	2.76	225.7 \pm 11.3	ND	225.7	622.9
F (Ext-3)	2.76	256.3 \pm 12.5	ND	256.3	707.4
G (Ext-4)	2.76	248.7 \pm 7.7	ND	248.7	686.4
H (Ext-5)	2.76	234.9 \pm 11.8	ND	234.9	648.3
I (PlayG-1) ^a	6.04	25.8 \pm 1.9	88.3 \pm 19.3	114.1	689.2
J (PlayG-2) ^a	6.04	33.4 \pm 2.5	149.5 \pm 5.9	182.9	1104.7
K (AirW-1)	1.48	ND	207.8 \pm 12.1	207.8	307.5
L (BlueB-1)	2.89	295.6 \pm 11.2	133.6 \pm 8.3	429.2	1240.4
M (Lot-1) a	4.71	ND	ND	ND	ND
N (Lot-2) a	4.71	ND	ND	ND	ND
O (Lot-3) a	4.71	ND	ND	ND	ND

a: Without labeling of antioxidants in ingredients.

b: Average of triplicate analyses.

c: Total antioxidant contents ($\mu\text{g/piece}$) = (BHT + BHA) \times weight/piece.

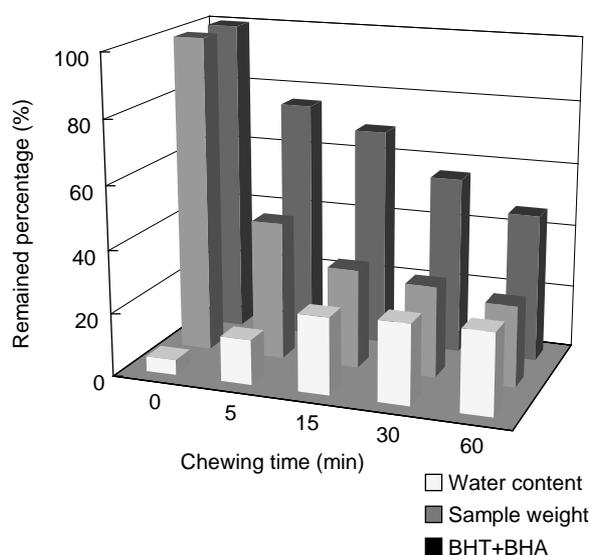


Figure 6. Changes of water content, sample weight and BHT and BHA contents remained in chewing gum during chewing.

VI. BHT and BHA Contents in Commercial Chewing Gum Samples

The quantitation results of BHT and BHA, using the study method, in 15 commercial chewing gum samples were shown in Table 4. The BHT and BHA contents in 15 samples were in the range of 0~296 and 0~208 $\mu\text{g/g}$, respectively, and the total antioxidant contents, BHT + BHA, was in the range of 0~429.2 $\mu\text{g/g}$. The total antioxidant content in a piece of chewing gum was in the range of 0~1313.9 μg . According to CNS chewing gum sanitary standard, the allowable BHT and BHA content in chewing gum and bubble gum was below 0.75g/kg (750 $\mu\text{g/g}$). In

this study, the BHT and BHA contents in 15 commercial chewing gum samples reached around half of the standard amount. No violation was found. No antioxidants were labeled in 5 out of 15 chewing gum samples; but, among them, 2 were identified with BHT and BHA in the range of 114.1~182.9 $\mu\text{g/g}$. Three in the other 10 samples were labeled with BHT adulteration, but both BHT and BHA were identified. The mislabeling of chewing gum still exists.

The above results indicate that a piece of chewing gum contains 0~1313.9 μg of BHT and/or BHA. Although BHT and/or BHA in the 15 samples dose not exceed CNS standard, suppose a 10~15 kg weight child takes 10 pieces of chewing gum in a day, the total amount of BHT and/or BHA consumed will be 6.5 mg (13 mg \times 0.5). If we consider the contents of BHT and/or BHA from other foods, the total daily consumption of BHT and/or BHA might exceed the allowable daily intake (ADI) of BHT (0.3 mg/Kg) and/or BHA (0.5 mg/Kg)⁽²⁷⁾ (3.0-7.5 mg per day for a child who is at the weight of 10-15 kg).

CONCLUSIONS

When 1 g chewing gum sample is extracted with 5 mL diethyl ether, and sonicated for 5 min after addition of 0.2 mL of 0.5% (w/v) 14OH ethyl acetate solution, the sample will be thoroughly degraded. 0.1 μL sample solution in diethyl ether layer is then injected into a GC for analysis. This is a simple and rapid quantitation method for analysis of BHT and BHA in chewing gum samples, which takes 15~20 min to complete a sample analysis. The recovery of BHT and BHA, in adulteration-recovery study, is 99~101% and 94~99%, respectively, with CV less than 8.6%. When

the solvent extraction and GC method was applied to analyze the BHT and BHA contents, no over-addition of BHT and/or BHA in commercial chewing gum samples was found. The average amount of BHT and/or BHA in commercial chewing gum samples was around half, 750 $\mu\text{g/g}$, of CNS standard.

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