Journal of Undergraduate Chemistry Research, **2021**, 20(2), 27

EVALUATION OF CHEWING GUM AND CEREAL PRODUCTS AS A POTENTIAL SOURCE OF BUTYLATED HYDROXYTOLUENE (BHT) TOXICITY IN CHILDREN

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Abstract

Butylated hydroxytoluene (BHT) is an antioxidant that reduces the oxidation rate in food products. Ingestion of BHT is of concern because studies on animals have shown that increased cancer rates and liver enlargement can occur with routine ingestion. To address toxicity concerns, the World Health Organization (WHO) has established a 300μg/kg·bw maximum daily intake level for BHT. This study was undertaken to assess the potential for BHT toxicity in children from the consumption of commercial cereal and chewing gum. BHT levels in these products were determined by solvent extraction followed by gas chromatography/mass spectrometry (GC/MS). The BHT levels in cereal ranged from 3.9 to 48.5 μg/g and 49.5 to 199 μg/g for chewing gum. Taking in to consideration the average body weight of toddlers, children, pre-teens, and teens, the results suggest that these products will not result in over exposure when consumed in moderation.

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Keywords: Gum, Cereal, BHT, Toxicity, Children, GC-MS

Introduction

Oxidation is a serious problem in the food industry due to its effects on the quality of food. Lipid oxidation affects the flavor, color and texture, odor of foods, and reduces the nutrients.[1] Butylated hydroxytoluene (BHT) is a lipophilic organic compound, a chemical derivative of phenol, that is useful for its antioxidant properties.[2] The Federal Drug Administration (FDA) regulates the quantity of BHT that can be used as a food additive to prevent food from oxidizing and becoming rancid. The FDA limit for gum bases is 1000 μg/g and 50 μg/g for dry cereal.[3] The FDA's Select Committee on Generally Regarded as Safe Substances (SCOGS) concluded "While no evidence in the available information on BHT demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted."[4] Consumers worldwide are beginning to scrutinize food products ingredient by ingredient. BHT use in foods generates a range of opinions in the popular press regarding food safety. Some say, because BHT is regulated by the FDA therefore it is safe. Others argue that even though they may be allowed by the FDA, there are still adverse effects on the human body, especially children. [5-10] To address some of these concerns, the World Health Organization (WHO) set the acceptable daily intake (ADI) for BHT at 300 μg/kg·bw. The ADI is the amount of a food additive, expressed as mg/kg body weight, that can be ingested daily over a lifetime without incurring any appreciable health risk.[11]

To date most studies addressing the BHT levels in chewing gum and cereal have been focused on method development and involve limited number of samples[11-16] with only one fairly recent study (2007) that addressed BHT intake levels of children.[17] To help address the growing public concern for the safety of these products, we analyzed 11 brands of chewing gum from 5 different manufacturers and 21 brands of dry cereal from 7 different manufacturers which listed BHT as a preservative. The goal was to determine the potential for toxic exposure (ingesting BHT in levels exceeding the ADI) in children from consuming these products.

Experimental

Materials

The 11 brands of chewing gum from 5 different manufacturers and 21 brands of cereal from 7 different manufacturers were purchased from local "BigBox" stores and supermarkets. Optima Grade acetonitrile, acetone, methanol, and ethyl acetate as well as the > 99% pure BHT and BHA standards were purchased from Fisher Scientific (Pittsburgh, PA). The 50mL glass extraction vessels with re-sealable screw on caps used for the extraction of BHT, were purchased from Milestone Inc. (Monroe Ct). Nylon syringe filters (0.45 μm x 47mm) and GC/MS auto sampler vials were purchased from Fisher Scientific (Pittsburgh, PA).

Instrumentation

Sample analysis was carried out on an Agilent 6890 GC equipped with an Agilent 5973 Mass Selective Detector equipped with a 30.0m x 250μm x 0.25μm DB5-MS fused silica capillary column (Agilent/J&W Scientific, Santa Clara, CA). The injection port was set at 250°C and the samples were analyzed using splitess injection. Helium was set to a constant flow at 1.5 mL/min. The initial column temperature was set at 100°C and then programmed from 100°C to 270°C at 20°C/min. The mass selective detector was operated in selective ion on monitoring using m/z 205 for BHT and m/z 165 for the Butylhydroxyanisol (BHA) internal standard.

Methods

Chewing Gum and Cereal Sample Preparation

Five random sticks or pieces of chewing gum were selected from the package and then cut into small pieces using a razor blade. The cut pieces were homogenized by mixing before sampling for analysis. Twenty grams of cereal was pulverized with a mortar then homogenized by mixing before sampling for analysis.

BHT Extraction Procedure

A 1.0 gram of homogenized sample (gum or cereal) was weighed into a 50mL glass vessel. 10 mL of the extraction solvent, containing 50ppm BHA as an internal standard, was added to the sample along with a magnetic stir bar. The vessel was capped

and extracted at room temperature with stirring for 30 minutes. After extraction, 1 mL of the extract was filtered through a 0.45µm Nylon syringe filter directly into a GC/MS autosampler vial for analysis. Triplicate analysis was performed on all samples.

Extraction Solvent Optimization

A random gum sample was selected to serve as the test sample. Triplicate samples were extracted using the procedure outlined above to determine which solvent had the best BHT extraction efficiency. The solvents tested were acetonitrile, acetone, methanol, and ethyl acetate.

Instrument Calibration

The stock solution of BHT was prepared by weighing 125 mg of >99% pure BHT into a 100 mL volumetric flask and filling it to the mark. This 1.25 µg/mL BHT stock was further diluted to prepare calibration solutions ranging from 2.5 to 50 µg/mL. The final calibration solutions each contained 50 ppm BHA as an internal standard. A representative calibration curve is shown in Figure 1.

Spike Recovery

Spike recoveries were performed to test the initial validity of the method. A random gum sample and a random cereal sample were spiked directly with 100 µL of a 500 µg/mL BHT stock, which resulted in each sample receiving a 50 µg BHT spike. The added BHT was allowed to absorb into the sample matrix for 15 minutes prior to extraction. Five spikes were performed on each sample for statistical analysis. The spike recovery for each sample was calculated using equation 1.[18]

$$\%\,\text{Recovery} = \frac{\mu g\ \text{of BHT}_{(\text{Spike})} - \mu g\ \text{of BHT}_{(\text{Sample})}}{50\mu g} \times 100 \qquad (1)$$

Results and Discussion

Before analyzing the actual chewing gum and cereal samples, we performed a simple method optimization and validation. Since the existing literature was unclear about the best extraction solvent for BHT, we choose to conduct a simple extraction solvent optimization first. Four potential extraction solvents (methanol, acetonitrile, acetone and ethyl acetate), were tested to see which had the best BHT extraction efficiency. These solvents were chosen for their ability to dissolve BHT and the inability to dissolve the gum or cereal matrix. It was determined that acetonitrile had the lowest BHT extraction efficiency, while methanol and acetone were 1.9 times more efficient then acetonitrile. Ethyl acetate was the most efficient solvent exhibiting 3.2 times solvating power of acetonitrile. A graphical representation of these results is shown in Figure 2. Based on these results, ethyl acetate was chosen as the extraction solvent for this study.

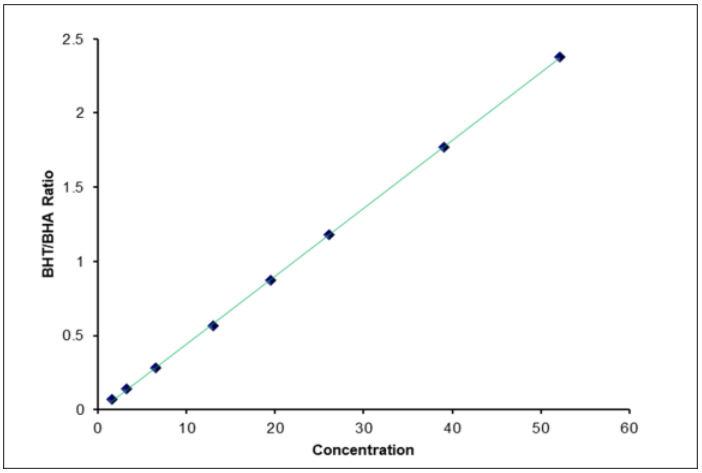

Figure 1. BHT calibration curve with BHA as an internal standard. Concentration in ug/mL. y = 0.0458x - 0.0168 R² = 0.9999

The proposed extraction procedure using ethyl acetate as the extraction solvent for GC/MS analysis, using selective ion monitoring, was validated by performing a spike recovery on a random gum and a random cereal sample. The average recovery for the cereal sample was 100.5 ± 7.0% and 97.1 ± 3.9 % for the gum sample. These nearly perfect spike recoveries validated our proposed methodology allowing us to proceed with the analysis of the actual samples.

The analysis results for the chewing gum samples are shown in Table 1 and the cereal samples results are shown in Table 2. Table 3 shows the maximum number of chewing gum and cereal

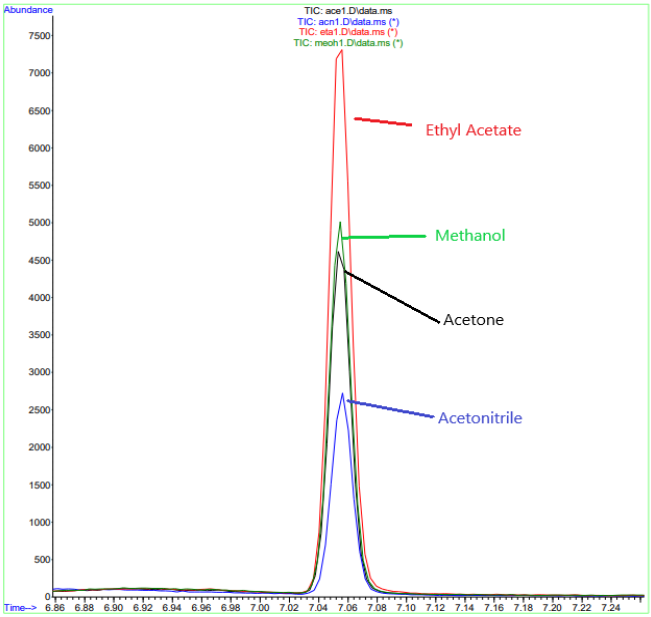

Figure 2. Overlay of GC/MS chromatographs for the extraction of BHT from chewing gum with Methanol, Acetonitrile, Acetone and Ethyl Acetate.

Table 1. The average BHT concentrations in the chewing gum samples that were tested.

		Average Concentration (µg/g)[a]	Serving Size (g)	Average Concentration per Serving (µg)[a]
Manufacturer A	Brand 1	143.2 ± 0.9	6.0	859 ± 5
Manufacturer B	Brand 1	68.7 ± 5.2	2.7	186 ± 14
	Brand 2	98.2 ± 1.3	2.7	251 ± 3
	Brand 3	107.9 ± 6.5	2.7	289 ± 17
	Brand 4	49.5 ± 1.3	2.7	134 ± 4
	Brand 5	81.0 ± 8.6	2.7	219 ± 23
Manufacturer C	Brand 1	197.2 ± 1.9	2.3	441 ± 4
	Brand 2	117.8 ± 4.7	8.0	942 ± 38
Manufacturer D	Brand 1	119.0 ± 1.5	6.0	714 ± 9
Manufacturer E	Brand 1	198.6 ± 9.7	2.0	397 ± 19
	Brand 2	162.8 ± 7.5	3.0	489 ± 23

[a]Error expressed as standard deviation (n=3)

servings per age group that can be consumed per day to stay below the 300 µg/Kg·bw recommended ADI value.

Conclusion

All the cereal and chewing gum samples tested were all below the FDA limits 50 µg/g and 1000 µg/g respectively. Based on the recommended 300 µg/Kg·Bw average daily intake, children from toddler to teen are in no serious threat of becoming overexposed to BHT from moderate consumption of cereal and gum products.

Acknowledgements

This research was conducted in conjunction with CSU's Center for Microwave Enhanced Chemistry (CMEC). Funding provided by: U.S. Department of Education Award P382A150041, NSF Award 0820958, DOD awards W911NF-07-0595 and W911NF-05-1-0407.

Table 2. The average BHT concentrations in the cereal samples that were tested.

		Average Concentration (µg/g)[a]	Serving Size (g)	Average Concentration per Serving (µg)[a]
Manufacturer A	Brand 1	48.5 ± 0.2	37	1795 ± 7
	Brand 2	5.9 ± 0.1	38	224 ± 4
	Brand 3	10.2 ± 0.3	43	439 ± 13
Manufacturer B	Brand 1	12.8 ± 0.4	60	786 ± 24
	Brand 2	8.2 ± 0.2	28	230 ± 6
	Brand 3	29.0 ± 1.5	32	870 ± 48
Manufacturer C	Brand 1	5.6 ± 0.2	40	224 ± 8
Manufacturer D	Brand 1	11.2 ± 0.5	20	224 ± 10
	Brand 2	26.1 ± 0.4	28	731 ± 11
	Brand 3	19.1 ± 0.7	28	535 ± 20
	Brand 4	25.5 ± 1.1	42	1071 ± 46
	Brand 5	27.0 ± 0.2	29	783 ± 6
	Brand 6	10.2 ± 1.0	32	326 ± 32
Manufacturer E	Brand 1	3.9 ± 0.1	40	156 ± 4
Manufacturer F	Brand 1	19.6 ± 0.5	31	608 ±16
	Brand 2	9.4 ± 0.1	30	282 ± 3
	Brand 3	5.0 ± 0.1	31	155 ± 3
Manufacturer G	Brand 1	20.7 ± 0.4	28	580 ±11

[a]Error expressed as standard deviation (n=3)

Table 3. Number of servings a day, per age group, to stay below the 300 ug/kg·bw allowance. Calculated using the average µg per serving from Tables 1 and 2.

Age Range (years)	Average Body Weight (kg)		Maximum Number of Servings per Day			
			Gum		Cereal	
	Female	Male	Female	Male	Female	Male
2-4	10.7	12.5	≤ 6	≤ 7	≤ 7	≤ 8
5-9	26.8	26.3	≤ 15	≤ 15	≤ 18	≤ 18
10-12	43.1	41.8	≤ 24	≤ 23	≤ 29	≤ 28
13-18	60	65	≤ 34	≤ 36	≤ 40	≤ 43

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