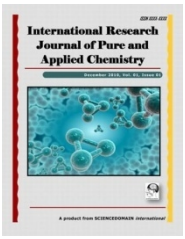

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Reversed Phase HPLC-UV Quantitation of BHA, BHT and TBHQ in Food Items Sold in Bindura Supermarkets, Zimbabwe

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Authors' contributions

This study is a collaborative effort of all the authors. Author PD designed the experiments and drafted the first draft manuscript. Authors PD and CM managed the analyses in the laboratory. Authors DS and CM read and corrected the draft and managed literature. All the authors finally read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine levels of Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tertiary butyl hydroquinone (TBHQ) in food items sold in supermarkets in Bindura town, Zimbabwe.
Study Design: Reversed phase HPLC-UV quantitation.
Place and Duration of Study: Department of Chemistry, Bindura University, November 2013 to March 2014.
Methodology: Twenty food items were bought, comprising of 6 vegetable oils, 7 bread spreads (3 butters and 4 margarines), and 7 snacks from local supermarkets around Bindura town. Food samples (10 g) were extracted using methanol/acetonitrile (100mL) mixture (1:1, v/v) by ultrasonication for 15 min, vortexing and centrifugation at 3000 rpm for 10 min. The contents were then filtered through 0.45µm Millipore filters and analyzed on a Varian HPLC equipped with a Rodyne manual injector with a 20 mL loop and a UV detector, prostar 325.

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Results: BHA was not found in all the samples. Levels of TBHQ ranged from 18.7±1.6 - 171.1±0.8 mg/kg while BHT ranged from 9.8±2.2 - 67.3±3.1 mg/kg. One sample from Zimbabwe consisted of the highest amount of TBHQ. Of the 7 butter/margarine types that were analyzed all of them consisted of BHT which ranged from 10.4±0.9 to 158.6±3.1 mg/kg while TBHQ was only found in South African samples. It ranged from 153.1±0.1 - 180.3±3.67 mg/kg. TBHQ was also found in higher levels for snack samples from South Africa. Only one sample from Zimbabwe consisted of TBHQ.
Conclusion: The present study has revealed that synthetic antioxidants are being widely used in food items at different levels thereby scoring the need to come up with local legal limits to regulate food industry products. TBHQ a banned antioxidant in European countries is still being used in Zimbabwe and South Africa although some analyzed food items did not reveal the identity of the antioxidant. Food manufacturers should be encouraged to reveal names and quantity of antioxidants added so as to ensure food safety.

Keywords: Synthetic antioxidants; Butylated Hydroxyanisole (BHA); Butylated Hydroxytoluene (BHT); Tertiary Butyl Hydroquinone (TBHQ).

1. INTRODUCTION

Antioxidants can be generally defined as substances that are added in packaged foods to improve shelf life by preventing rancidity (formation of unpleasant adours), browning, and loss of fresh taste or texture [1,2]. Antioxidants have achieved wide applications in many food products such as fruit juices, chocolate products, meats, sweets and snacks. Recent studies indicated that though synthetic antioxidants are often present in different food items, manufacturers and advertisers generally never discuss them [3]. Thus, consumers are often unaware of their existence and possible health risks.

Synthetic antioxidants are chemically synthesized since they do not occur in nature and their presence in foods have received mixed feelings by the scientific community [4,5]. Important examples include Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), Tertiary butyl hydroquinone (TBHQ), propyl gallate (PG), dodecyl gallate (DG) and octyl gallate (OG) [6]. Scientific studies have proved that they can have either harmful or beneficial effects to the human body [7]. Beneficial effects include inhibition of bacterial growth and formation of harmful chemicals, antiviral activity, anti-inflammatory action and protection of sperm membrane from cold shock [8]. Studies have also shown that if they are used within the recommended levels, most synthetic antioxidants have shown to be effective in preventing spoilage of food. In fact, at these low concentration levels, synthetic antioxidants have been observed to add beneficial effects to human health such as prevention of cancers [9]. It is important to point that the adherence to recommended levels lies on the honest and sincerely of the manufacturer. Food and Drug Administration regulatory bodies only define antioxidants as dietary supplements therefore antioxidants are always perceived in solely a positive manner. It has been shown that manufacturers tend to add more in order to gain maximum benefits [10]. More often food manufacturers do not reveal names of antioxidants that have been added. Also recommended levels are usually for a single antioxidant and not for binary or ternary mixtures which are often used. Almost anything that is eaten these days consist of one or more antioxidants therefore there is a higher probability that we are exceeding the safety limits. Even though at current recommended levels of intake synthetic antioxidants may seem to pose no reasonable threat to health, however long term chronic

ingestion of them may aid in modifying the acute toxicity of other harmful chemicals we consume everyday leading to chronic side effects [11]. Harmful effects that may develop include, development of cancers, suppression of the immunity by shortening the life cycle of cells that participate in the prevention of diseases in the human body, increase in organ weight and development of chromosomal and sperm abnormalities [12]. A recent study carried out with rodents and monkeys has shown that synthetic antioxidants might exert toxic side effects [13]. Both BHT and BHA have been observed to exert enzyme or lipid alterations or carcinogenic effects [14]. Their degradation products have been implicated in the etiology of carcinogenic effects by causing damage to the human DNA [15].

Some countries have taken heed to these limitations of synthetic antioxidants and are now banning and placing a restriction on their use [16]. The use of TBHQ in foods is no longer allowed in European countries, Canada and Japan [16]. In Zimbabwe most snacks such as, potato crisps and zap nacks, food items such as oils, meats and milk products may consist of TBHQ, BHA and BHT. BHA and BHT are banned in Japan [16]. In other countries such as USA strict adherence to recommendation limits is enforced [17]. Food industries are forced to state names of antioxidants present in their food products and to declare the reason for adding such antioxidants. These regulations are not enforced in Zimbabwe. Synthetic antioxidants are present in many food items. Some of the food items in the market do not reveal names of antioxidants present.

It is apparent therefore that periodic monitoring of food substances to check adherence to recommended levels is very crucial. This study therefore was designed to determine levels of BHT, BHA and TBHQ in selected food items that are sold in supermarkets around the city of Bindura.

2. MATERIALS AND METHODS

Standard antioxidants, Butylated hydroxyanisole (98%), Butylated hydroxytoluene (98), Tertiary butyl hydroquinone (97%) and HPLC solvents (methanol, acetonitrile) were bought from Merck (Darmstadt, Germany). All other chemicals were analytical grade purchased from SkyLabs, South Africa

2.1 Food Samples

Twenty three food items were collected, comprising of 6 vegetable oils, 7 bread spread (3 butters and 4 margarines) and 7 snacks. Samples were purchased from local supermarkets around Bindura town. All samples, with the exception of cooking oils, were stored in a refrigerator before analysis.

2.2 Preparation of Standard Stock Solutions

A stock solution containing 500 mg ml^{-1} of each of TBHQ, BHA, and BHT) were prepared in methanol/acetonitrile solution (1:1, v/v). The flask was shaken to homogenous the sample. The flask was covered with aluminum foil and stored in a freezer (4 ^{o}C) away from light. Standard working solutions were then prepared by diluting appropriate amounts of the stock solution in methanol/acetonitrile (1:1, v/v).

2.3 Extraction of Antioxidant from Food Samples

Food samples (10 g) were placed into a 100 ml stoppard conical flask. Methanol/acetonitrile (100mL) mixture (1:1, v/v) was added, and contents ultrasonicated for 15 min. The mixture was then vortexed and centrifuged at 3000 rpm for 10 min. The contents were then filtered through 0.45µm Millipore filters and analyzed on an HPLC [18].

2.4 HPLC Analysis

Chromatographic analysis was performed on a Varian HPLC equipped with a Rodyne manual injector with a 20 mL loop and a UV detector, prostar 325. The detector was controlled remotely by the Varian Star/ Galaxie Chromatography Workstation software version 6. All the analytes were separated using HPLC Varian Microsorb MV 1005 packed C18 columns 250 x 4.6 mm id, 5 µm particle size, 100 Å SPELCO. The mobile phase consisted of methanol/acetonitrile/water with 1% acetic acid (1:1:3) (pH 2.95). The mobile phase was filtered and degassed using ultrasonic agitation. The separation mode was isocratic and the flow rate was kept at 1 mL/min. the injection volume was 10 µL. The UV detector was fixed at 280 nm. Six standards solution of BHA, BHT, and TBHQ in acetonitrile of concentrations of 1, 10, 25, 50,100, and 300 mg/L were prepared. Calibration curves of peak area against the concentration of standard antioxidants were used for quantification.

3. RESULTS AND DISCUSSION

3.1 Validation Parameters

Calibration curves R^2 were above 0.996 for all standards. Regression equations were y =13245x + 2657, y = 14550x - 7561, y = 13672x - 4358 for BHT, BHA and TBHQ respectively. The limits of detection were 0.132, 0.117 and 0.121mg/kg for BHT, BHA and TBHQ respectively. Percentage recovery ranged from 74.5-89.9%. A typical chromatography obtained by analyzing standard antioxidants (50 mg/L) is shown in Fig 1 below; Chromatograms recorded were free of interfering peaks. Peak purity assessed by the Varian Star/ Galaxie Chromatography Workstation software version 6 revealed that all peaks purity levels were equal to or greater than 98%.

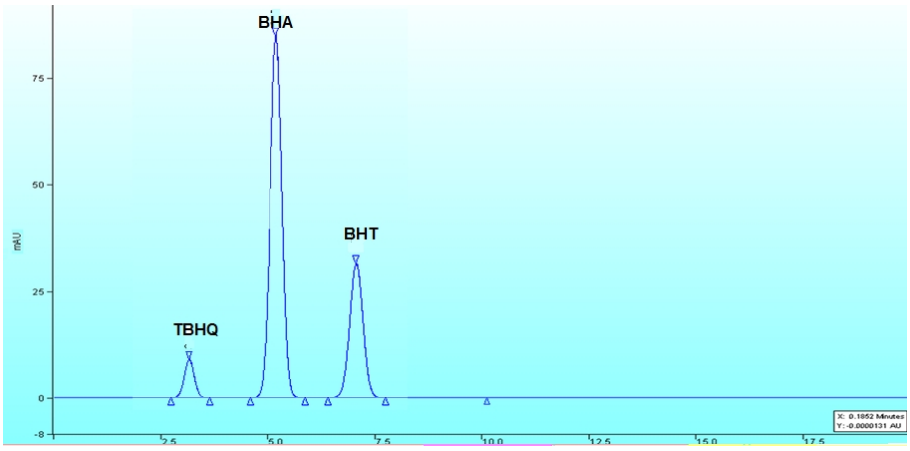

Fig. 1. A typical chromatogram for standard antioxidants; BHT, BHA and TBHQ

3.2 Analysis of Food Items

Although synthetic antioxidants can help in increasing the shelf life of many food items long term abuse cannot be overruled thereby pointing to the need to carry out routine analysis to check for maximum limits adherence. In many countries especially developing countries these checks are unavailable. In such a situation research studies are necessary to screen for synthetic antioxidants. This study analyzed levels of BHT, BHA and TBHQ in food items sold in supermarkets around Bindura town. Food items that were found to contain synthetic antioxidants are shown in Tables 1-3. Manufacturing country was as listed on container labels. All the samples were analyzed using reversed phase high performance chromatography coupled to ultraviolet detection. Peak identification of samples was based on the comparison with retention times of standard compounds peaks and were confirmed by spiking known standards to the sample. The external standard method was used for quantitation using calibration curves fitted by linear regression analysis. Six cooking oil samples two manufactured from Zimbabwe and four from South Africa were analyzed. It was observed that BHA was not found in all the samples. Levels of TBHQ ranged from 18.7 to 171.1 mg/kg while BHT ranged from 9.8 to 67.3 mg/kg. One sample from Zimbabwe consisted of the highest amount of TBHQ. Of the 7 margarine types that were analyzed all of them consisted of BHT which ranged from 10.4 to 158.6 mg/kg while TBHQ was only found in South African samples only. It ranged from 153.1 to 180.3 mg/kg. TBHQ was also found in higher levels for snack samples from South Africa. One sample from Zimbabwe consisted of TBHQ. It is interesting to note that for all the samples that were found to consist of synthetic antioxidants most of them do not violate the legal limit of 200 mg kg^{-1} for total synthetic antioxidants as specified in the Food Act and Regulations [19] except one sample manufactured in Zimbabwe. According to Limits for antioxidants in edible oils in Europe and US TBHQ is no longer permitted as a food additive while BHT should not exceed 100mg/kg [20]. For most of the analyzed samples, the manufacturers declared the presence of synthetic antioxidants, but they did not declare the type or its level.

Table 1. Levels of synthetic antioxidants in selected oils

Manufacturing country	Synthetic antioxidants (mg/kg)		
	TBHQ	BHA	BHT
Zimbabwe	20.2	ND	9.8
Zimbabwe	171.1	ND	ND
South Africa	18.7	ND	36.4
South Africa	33.6	ND	14.3
South Africa	47.1	ND	44.2
South Africa	88.3	ND	67.3

ND = not detected

Table 2. Levels of synthetic antioxidants in selected butter and margarine

Manufacturing country	Synthetic antioxidants (mg/kg)		
	TBHQ	BHA	BHT
Zimbabwe	ND	ND	130.6
Zimbabwe	ND	ND	100.4
Zimbabwe	ND	ND	158.6
Zimbabwe	ND	ND	121.6
South Africa	160.3	ND	7.8
South Africa	180.3	ND	17.8
South Africa	153.1	ND	10.4

ND = not detected

Table 3. Levels of synthetic antioxidants in selected starch based snacks

Manufacturing country	Synthetic antioxidants (mg/kg)		
	TBHQ	BHA	BHT
Zimbabwe	160.7	ND	31.6
Zimbabwe	ND	ND	106.2
Zimbabwe	ND	ND	151.3
Zimbabwe	ND	ND	21.9
South Africa	180.7	ND	7.3
South Africa	180.5	ND	1.8
South Africa	167.4	ND	3.3

ND = not detected

4. CONCLUSION

The study revealed that food industries in Zimbabwe and South Africa still use TBHQ as an antioxidant in food items despite its being banned in other countries. Levels of BHT exceeded EU and US total limit of 100mg/kg however most of the levels are within the total legal limits (200mg/kg) of other countries like Malaysia. It is important for industrialist in Zimbabwe to adopt one legal limit so as to prevent confusion and abuse. No BHA was detected in all the samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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