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RESEARCH ARTICLE

Detection and determination of Butylated Hydroxyl Toluene (BHT) in imported milk for adults and infants at Baghdad city

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Abstract

To prove there were using Butylated Hydroxyl Toluene (BHT) in food as an additive to conserve food, this study involved a test and analysis of (22) samples of imported milk, which taken from a local markets at different places from Baghdad city. This study aimed to detect and determine the concentration of (BHT) in milk, an obtained results showed a presence of (BHT)in seven samples from (22) samples of milk are contain allowed limits of (BHT) due to the results of High Liquid Performance Chromatography (HPLC) and Ultra violet (UV) test. This detected limits of (BHT) as a food conserving, as not more than (75) mg/kg, According to Gulf Safety Organization standard (GSO), Food and Agriculture Organization (FAO) and World Health Organization (WHO).

INTRODUCTION

Butyl Hydroxyl Toluene (BHT) scientifically is named Alpha phallic organic compounds, chemically is a derivative of phenol (2, 6-di-tert-butyl-4-methylphenol).Due to it's a property as antioxidant, (BHT) used as conserving in food. The regulations of European, U.S.A and other countries in different places of the world, allow a small percent of (BHT) to use as a food additive (Soberón et al., 2006; Tavassoli and Djomeh, 2011).And other chemical properties which make it an excellent preservative, and it's also may be implicated in health effects (Bauer et al., 2005). Some times (BHT) appear alone in a food but are often used in combination with other chemicals that also have an antioxidant activity including Propyl gallate, Citric acid, Phosphoric acid and Ascorbic acid (Weber, 1997). Widely, Chemicals are used in food industries wherever oxidation in fluids (e.g. Fuel, Oil) and other materials were they must be treated, in addition the free radicals must be kept in check in food. Several types of food items at markets do not reveal names of antioxidants present. (BHT) founds in many types of food such as butter, meats, cereals, milk, chewing gum, baked goods, snack foods, dehydrated potatoes and beverages. Also used to preserve food odor, color and flavor (Race, 2009). Also to reducing power of extracts by increasing their concentrations (Chen et al., 2013). Due to a low cost of (BHT), on another hand, due to its stability and easy to obtain, commonly it's used as anti-oxidant in food and milk products, Fats must be preserved by anti-oxidizing agents to prevent rancidity (Hsui-Jung et al., 2003). (BHT) acts as an anti-oxidant in order to maintain the proper flavor and odor in food including chewing gum, milk and other foods (Witter, 2005). (BHT) is used widely as preservative in food and in milk products which prevents the oxidation of lipids. And other countries most snacks such as, potato crisps and zap, food items such as oils, meats and milk products may consist of Tertiary-butyl hydroquinone (TBHQ), Butylated hydroxyl anisole (BHA) and Butylated hydroxyl toluene (BHT) (Van Esch, 1986).There is nothing 'natural' about (BHA), (BHT), and (TBHQ). They are synthetic compounds produced in laboratories. (BHT) was initially developed as an antioxidant for use with petroleum and rubber products (EFSA, 2004).Synthetic antioxidants are frequently found in animal feed and not just as antioxidants. For example, some

people think that (BHT) protects against Aflatoxin in Turkey and so it is added to their food (Klein, and van Vleet, 2003).

This substance is used in low concentrations as a stabilizer/antioxidant in many types of products Because (BHT) is not so dangerous for health and is added in small amounts, which means that it does not have to be declared to the Products Register, the quantity of (BHT) entered in the register is clearly an underestimate of the amount of the substance used in most of countries(Day, 2003), but most reviews of the scientific studies acknowledge that there may be some problems with them but the benefits, to us as consumers, outweigh the small potential of risks(EFSA, 2004).Some countries have taken heed to these limitations of synthetic antioxidants which they at this time are banning and placing a restriction on their use. Adding of (BHT) in foods is no longer allowed in European countries, Canada and Japan (Van Esch, 1986).

In other countries such as USA strict adherence to recommendation limits is enforced (Bauer, 2005). Food industries are forced to state names of antioxidants present in their food products and to declare the reason for adding such antioxidants. Food and Drug Administration regulatory bodies' only define antioxidants as dietary supplements therefore antioxidants are always perceived in solely appositive manner. It has been shown that manufacturers tend to add more in order to gain maximum benefits (Gharavi et al., 2007).The oxidative characteristics and Metabolites of (BHT) may contribute to carcinogenicity (EFSA, 2012). (BHT) seems to accumulate in Adipose tissue (Fatty tissue) with lower levels found in the liver. It takes about 7-10 days to leave body, once a person has stopped ingesting it; however, one study showed that only 68% had been excreted within 11 days. We will need to try avoid them for at least 2-4 weeks. Some positive results may occur quite quickly but others will take time to appear as your body needs to 'detox' itself (Race, 2009).

Materials and Methods

Milk Samples

A number of (22) milk samples are collected randomly, they are purchased from local supermarkets at Baghdad city, the trade names and origin also production and expire date shown in table (1). All samples are stored at room temperature before analysis.

Table (1): Trade Name, Origin, Date of production, Expiration Date of whole milk samples.

No.	Samples of Milk	Origin	Production Date	Expiration Date
1	Almarai	Oman	6/2013	6/2014
2	Dielac1 Milk (Powder)	Vietnam	4/2013	9/2014
3	Guigoz1 milk (powder)	Philippines	10/2012	4/2014
4	Smillaknyoshor milk	Aspin	7/2013	10/2014
5	Iysomil1 milk (powder)	Holland	7/2011	7/2013
6	Promyl gold milk	Ireland	3/2012	3/2014
7	Cerelac wheat and fruit	Aspin	10/2012	4/2014
8	Almudhish milk (Powder)	Switzerland	4/2013	10/2014
9	Nido compatible milk (powder)	Switzerland	10/2013	1/2015
10	Anchor milk (powder)	New Zealand	6/2014	5/2015
11	Novolac1 milk (powder)	Germany	4/2013	10/2014
12	S26gold milk (powder)	Ireland	1/2012	12/2013
13	Dielac2 Milk (Powder)	Vietnam	9/2013	9/2014
14	Bayoumal plus 1 milk (powder)	Belgium	1/2013	6/2014
15	Simillak milk (powder)	Ireland	1/2013	7/2014
16	Saudi Junior milk (powder)	Switzerland	6/2012	12/2013
17	Guigoz2 milk (powder)	Philippines	10/2012	4/2014
18	Norsoy milk (powder)	Ireland	6/2012	12/2013
19	Geen plus milk (powder)	Ireland	1/2013	7/2014
20	Novolac AC2 milk (powder)	France	10/2011	10/2013
21	Simillak gen plus milk (powder)	Ireland	1/2013	7/2014
22	Pook milk (powder)	Denmark	7/2014	2/2016

Preparation of Standard solution of (BHT)

A stock of solution from (BHT) is prepared by dissolving 1mg of (BHT) Standard in 100 ml of Acetonitrile a volumetric flask, and then standard solution was prepared as different concentration as a serial solutions (0.5, 1, 2, 4, 6 and 8) ppm from stock solution, by applying the equation **C1 x V1 = C2 x V2**

Samples Preparation

Samples are prepared from fine powder milk, after taken Ten gm of powdered milk, then placed in conical flask size 500 ml with rubber stopper, for each sample of milk separately.

A 100-mL of acetonitrile and methanol in ratio (1:1) was added to the flasks then degased by ultra-sonication for 15min. Then the mixture of acetonitrile and milk, shacked for 60 min and centrifuged at 3000 rpm for 10 min. at last contents are filtered with 0.45µm Millipore filters and analyzed by (HPLC).

HPLC Analysis

Chromatographic analysis was performed on a Varian HPLC (CECIL/England) equipped with a manual injector, a loop 50 µl and UV detector, wavelength 280 nm. all of analyses were separated using HPLC C18 columns 250 x 4.6 mm id, 5µm particle size, The mobile phase consisted of acetonitrile/water (9:1) The mobile phase was filtered and degassed using ultrasonic, the separation mode was isocratic and the flow rate was kept at 1ml /min. with injection volume 50µl,the UV detector was fixed at 280nm. A six standards solution of (BHT) in acetonitrile of concentrations start from (0.5, 1, 2, 4, 6, and 8) ppm. were prepared, the calibration curves of peak areas against concentration of standard antioxidants were used for quantification of (BHT) as shown in figure (1)

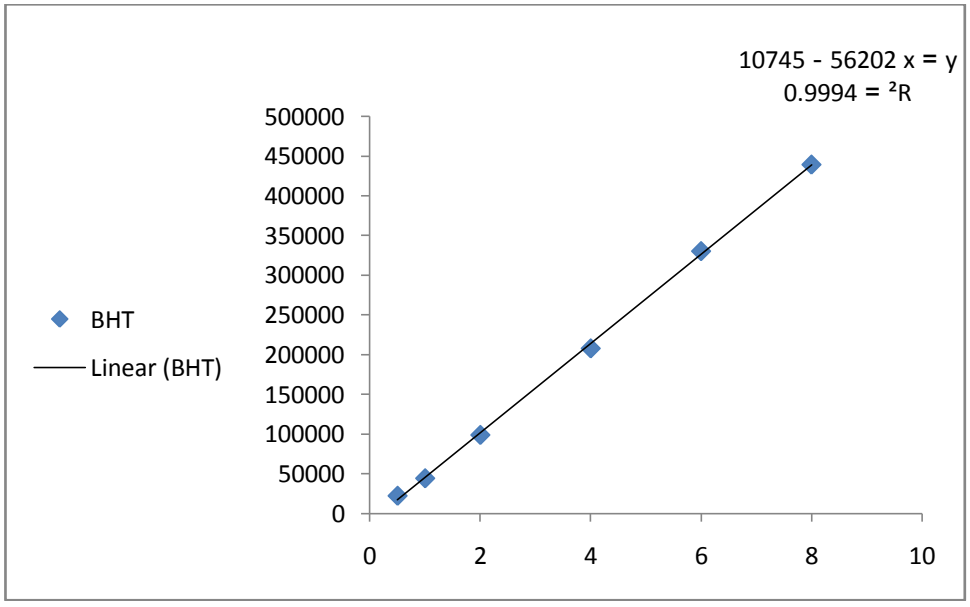


Figure (1): Graph of calibration curve of BHT

Results and Discussion

An obtained results from analysis of (BHT) by HPLC, showed a chromatogram of the peak for (BHT) standard, and the retention time of (BHT) Standard in 8.69 min. as shown in figure (2)

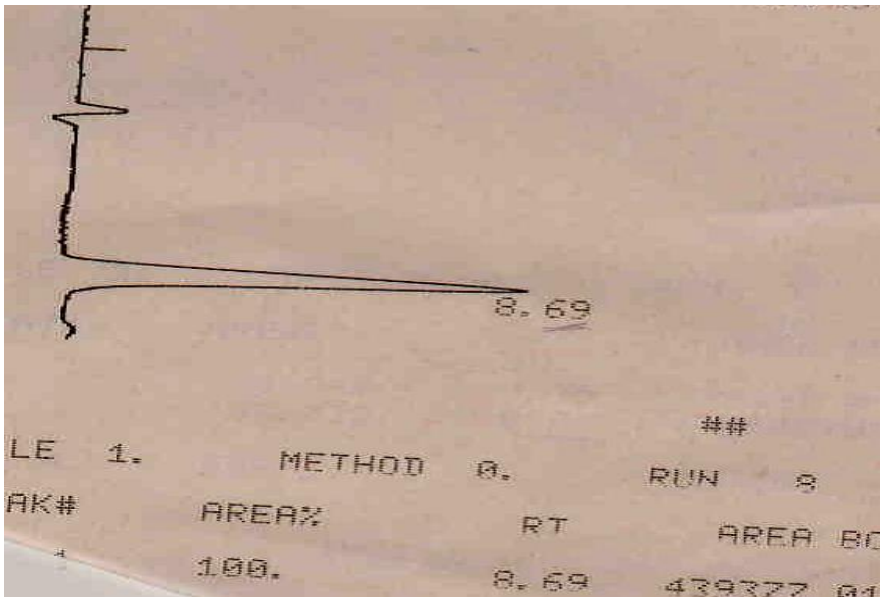
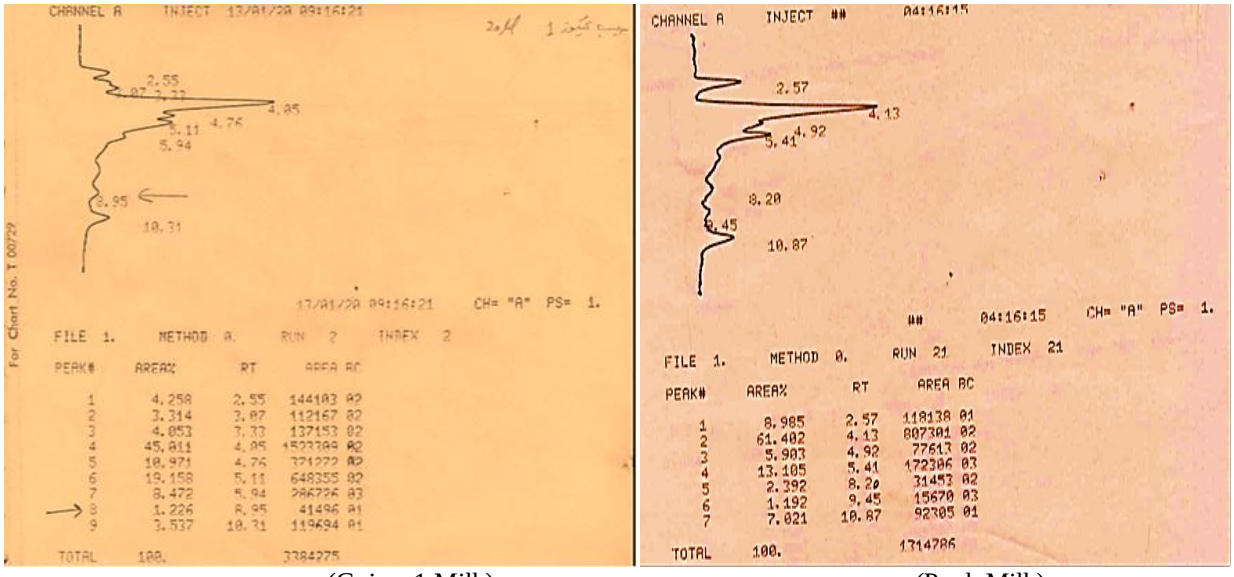


Figure (2): Graph of (BHT) concentration in standard

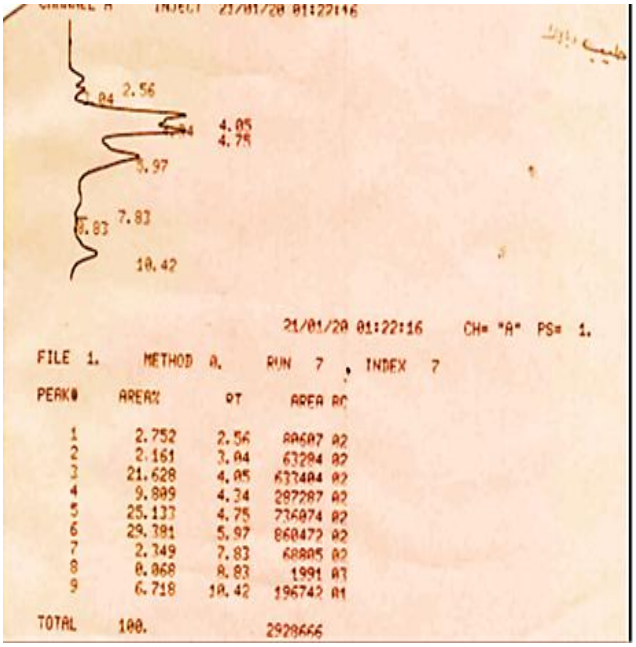
The concentration of (BHT) in milk, was achieved by extracting (BHT) from milk samples and comparing with the standard sample of (BHT) by a calibration plot, figure (1) displays the response ratio vs. the concentration of (BHT). The calibration plot was plotted due to applicable data that provided by HPLC, the X-axis simply is a known concentration by ppm units for (BHT) to standardized solutions, and Y-axis is a response factor, which defined as a peak area of (BHT) over the peak area was recorded by HPLC. The detection limits is recorded from (0.5) to (8) ppm. The purpose of this research, is to detect and determine the concentrations of (BHT) in (22) different samples of adults and infants milk. These concentrations of (BHT) in seven samples of milk and they were (0.3, 0.6, 0.7, 0.75, 1.25, 1.80 and 2) ppm. as shown in table (2),

an obtained results as figure (3, 4 and 5) shows a chromatogram of a four milk samples contain (BHT) compound.

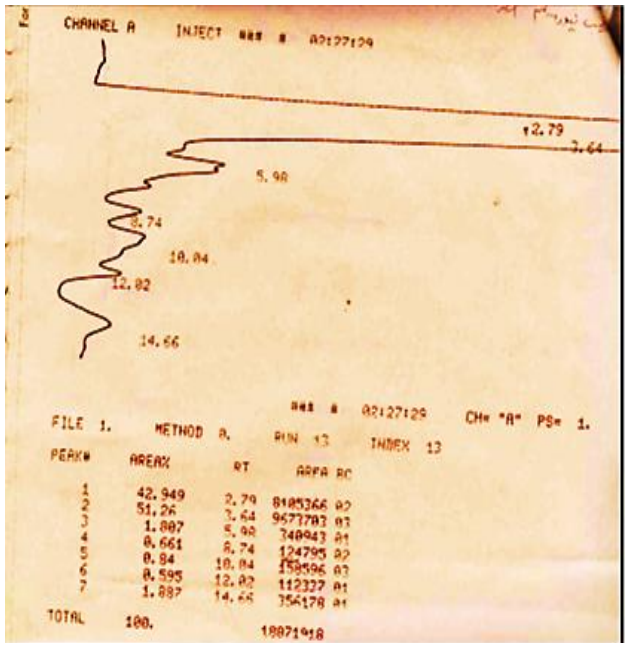


(Guigoz1 Milk) (Pook Milk)

Figure (3): Graphs showed some samples are in allowed limits

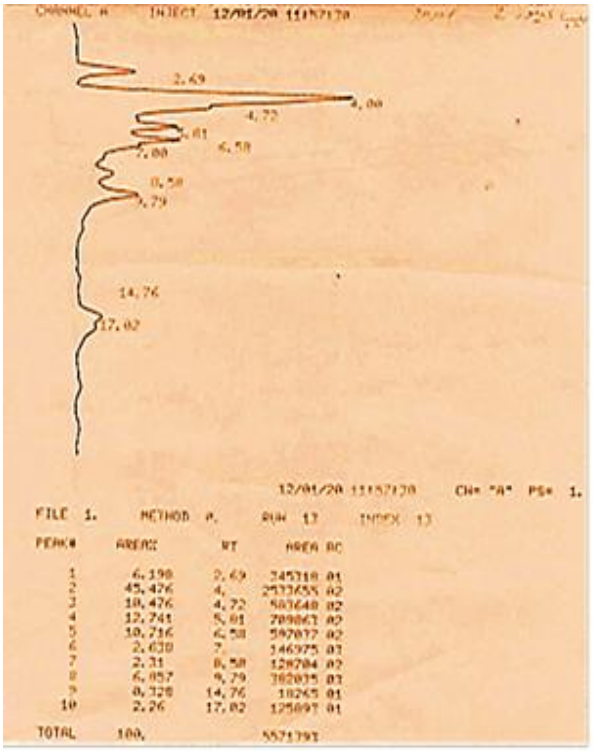


(DielacMilK**)**

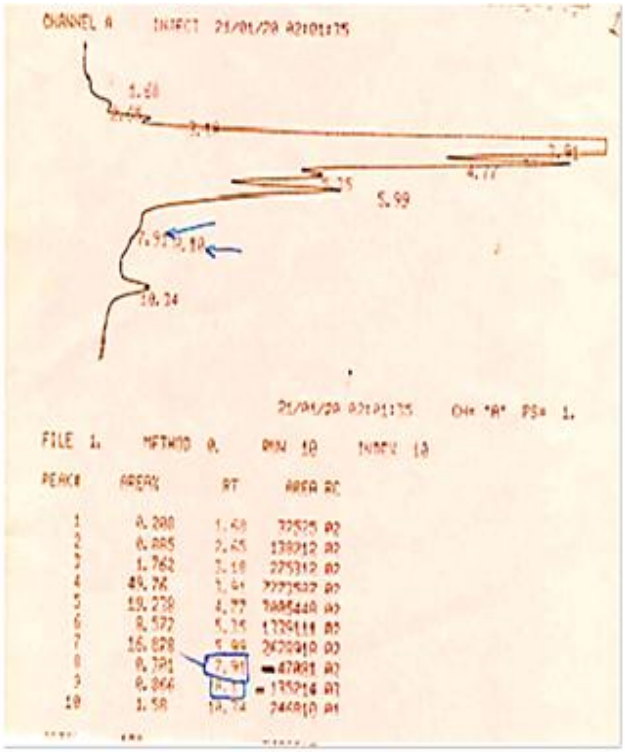


(Nido compatible Milk**)**

Figure (4): Graphs showed some samples are in allowed limits

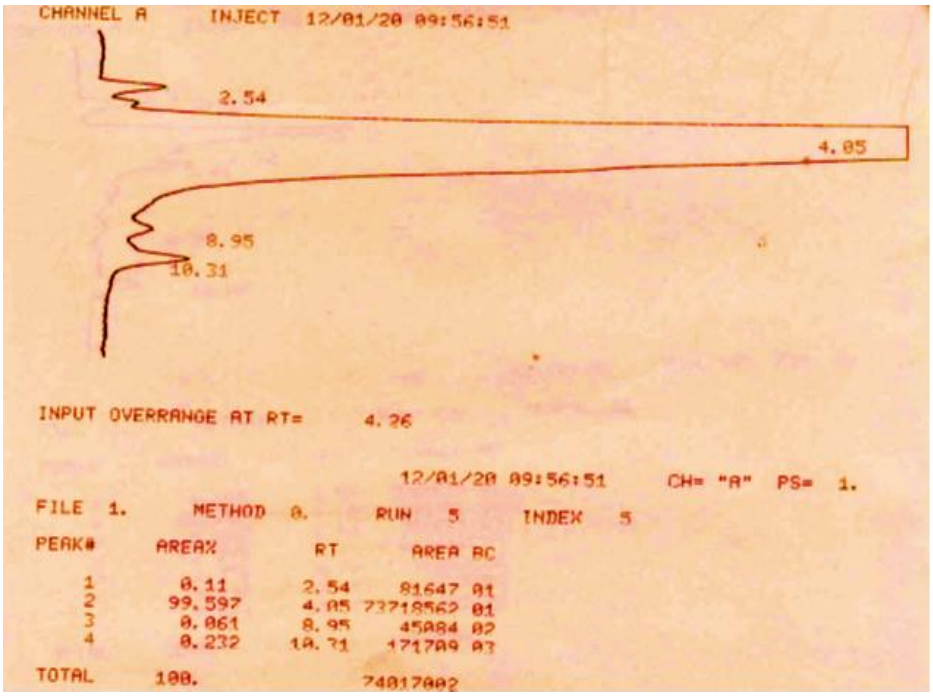


(Guigoz2Milk)



(Lysomil milk)

Figure (5): Graphs showed some samples are in allowed limits



(Saudi Junior Milk)

Figure (4): Graphs showed some samples are in allowed limits

Table (2): Results of detecting (BHT) compound in samples of whole milk

No.	Samples name	BHT concentration (ppm)	EMRL (ppm) (GSO)In BHT
1	Almarai	UDL	75
2	Dielac1 Milk (Powder)	0.6	
3	Guigoz1 milk (powder)	0.7	
4	Smillaknyoshor milk	UDL	
5	Iysomil1 milk (powder)	2	
6	Promyl gold milk	UDL	
7	Cerelac wheat and fruit	UDL	
8	Almudhish milk (Powder)	UDL	
9	Nido compatible milk (powder)	1.80	
10	Ankor milk (powder)	UDL	
11	Novolac1 milk (powder)	UDL	
12	S26gold milk (powder)	UDL	
13	Dielac2 Milk (Powder)	UDL	
14	Bayoumal plus 1 milk (powder)	UDL	
15	Simillak milk (powder)	UDL	
16	Saudi Junior milk (powder)	0.3	
17	Guigoz2 milk (powder)	1.25	
18	Norsoy milk (powder)	UDL	
19	Geen plus milk (powder)	UDL	
20	Novolac AC2 milk (powder)	UDL	
21	Simillak gen plus milk (powder)	UDL	
22	Pook milk (powder)	0.75	

***UDL under detection limits.**

After searching the references of this study, also another papers which not listed in this work, I found (Shasha et al., 2014), has mentioned about (BHT) concentrations in different types of food (butter, margarine and starch based snacks) the detection limits ranged (1.8 to158) mg/kg while (BHT) concentration in milk (infant, adult) Ranging from(0.3 to 2) mg/kg, Results showed some samples are in allowed limits, BHT should not exceed 100 mg/kg (Madhavi and singhal, 1996).Our detection limits were in limit of (BHT)in food 75 mg/kg. According to the GSO standard (Marekov et al., 2010).

Conclusions

This study proved in order to determine the concentrations of (BHT) in different samples of infant and adult milk. These concentrations in ten gram of milk was (0.3, 0.6, 0.7, 0.75, 1.25, 1.80, and 2) ppm and limits of (BHT) in food is 75 mg/kg, According to the Gulf safety organization (GSO) standard (Karovicova and Simko, 2000). Also World Health Organization (WHO) and Food Agriculture Organization (FAO), at 1987 they fixed the limits of (BHT) as 75 mg/kg (FAO, 1987). Where studies showed that increasing amounts of this (BHT) above allowed limits, can cause a cancer and enlargement of liver also affects the nervous system, but used it within the limits considered safe and no harm to human. There is also concern that (BHT) may convert to other substances in the human body that may be carcinogenic. For example, one conversion product of (BHT) (hydro peroxide form) has been shown to disrupt the chemical signals that are sent from cell to cell (Race, 2009; Haasand Levin 2006).

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