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CHEMICAL STUDY ON BENZENE CONTAMINATION OF SOME BEVERAGES IN EGYPTIAN MARKET

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ABSTRACT

Benzene is considered as a human carcinogen that normally exposed to human from environment. But low levels of benzene can be formed in beverages containing benzoate salts as an antimicrobial agent and ascorbic acid which may be either naturally present from a fruit juice ingredient in the soft drink or added as an antioxidant food additive. Benzene formed by decarboxylation of the benzoic acid salts in the presence of ascorbic acid (vitamin C). The purpose of this study was to estimate the benzene contamination in some beverages products in Egyptian, As well as studying the effect of different storage temperatures at different periods using different benzoic acid concentrations on benzene formation in some beverages. It was found fluctuation levels of benzene in the tested products from 7.8 to 22.7 ppb. Benzene contents were found to be depends on both benzoic and ascorbic acid contents where the higher levels of benzene were found at higher contents of both benzoic and ascorbic acids contents together. Formation of benzene was found to be increased when the products were stored for extended periods and at elevated temperatures, even at only 50 ^{o}C for three months. So, juices manufacturers should reformulate their products to eliminate benzene formation by using alternative preservatives of benzoic acid in high vitamin C drinks by other material such as sorbic acid.

INTRODUCTION

Benzene is one of the contaminants with the clearest evidence of carcinogenicity, and has been classified as carcinogenic agent to humans (Group 1) by the International Agency for Research on Cancer (IARC, 1987). It has been reported to cause leukemia and other blood-related disorders. Active as well as passive smoking, automobile exhaust, and driving or riding in automobiles are postulated as the most important pathways of benzene exposure. Since high levels of benzene metabolites are frequently reported among children and non-smoking workers without occupational exposure. (Johnson *et al.,* 2007) hypothesize that there may be significant sources of benzene, hitherto unidentified.

It was found that low levels of benzene can be formed in soft drinks containing certain food preservatives and nutrient additives. Beverages, especially those containing benzoate salts and ascorbic acid, are potential sources of contamination with benzene due to the decarboxylation of benzoate by a hydroxyl radical. Benzoate salts used as an antimicrobial agent in certain soft drink products and ascorbic acid may be either naturally present from a fruit juice ingredient in the soft drink or added as an antioxidant food additive. These reactions are catalyzed by trace levels of metal ions that reduce oxygen via reactions involving ascorbic acid to form hydroxyl radicals. However, there are no legal limits for the contamination of beverages with benzene, and the maximum contaminant level established for drinking water can be used. The World Health Organization (WHO) and the US Environmental Protection Agency (APA) established the limit for benzene in drinking water at 10 and 5 µg L^{-1}, respectively (Sanchez *et al.,* 2012).

The soft drink industry, working with the US Food and Drug Administration (FDA, 2014), found that when ascorbic acid was used as an ingredient along with sodium benzoate (a preservative), benzene formation could occur and this formation was exacerbated when the beverage was stored for extended periods. The latest study, volatile organic compounds in foods: a five year study was conducted by the FDA whereas benzene was found in all foods tested, including fruit and vegetables and these levels ranged from 1-190 ppb (ICBA, 2006).

In 2006, the Korea Food & Drug Administration (KFDA) announced that it had detected benzene in 27 out of 30 (90%) vitamin-enriched drinks on sale in South Korea. It said the detected amount of benzene – ranging from 5.7 to 87.8 ppb which was not harmful to humans but advised manufacturers of beverages containing more than 10 ppb of benzene to voluntarily recall their products (Patton, 2006).

This study aims to; firstly, monitor benzene contents in different types of juice samples in the Egyptian market with its correlation with benzoate and ascorbic acid contents. Secondly, studying the effect of different storage temperatures at different periods using different benzoic acid concentrations on benzene formation in soft drink.

Materials and Methods:

For the first part of this study, twenty different kind of samples have been chosen for this study which including four different kinds of juices and five different trade marks from different markets in Egypt. Three package samples have been analyzed for each chosen type. For the second part, orange juice has been prepared freshly for studying the effect of storage at different temperatures and benzoic concentrations.

Chemicals and Reagents for Vitamin C Analysis:

Vitamin-C (L-ascorbic acid AA), Extra pure, was supplied by E Mark, Darmstadt, F.R Germany. Methanol and acetonitrile were HPLC grade (Sigma Aldrich Germany). Potassium dihydrogen phosphate and meta phosphoric acid (MPA) are extra pure from Fluka (Switzerland).

Standard Preparation:

Ascorbic acid stock standard solution was prepared in distilled water and to prevent the loss of activity of standard solutions and extracted samples were protected from light using stopper amber flasks and stored at 4℃ in the dark.

Instrumentation and Chromatographic Conditions:

The HPLC system consists of the HPLC (Dionex 3000 system) equipped with two pumps P680 and Ultimate 3000 pump; auto injector / auto sampler ASI 100 Dionex syringe loading sample injector valve’s fitted with 20µl sample loop of 150 vials and UV-VIS Detector Chromatographic separation was achieved using Column ODS-3 C18 (GL Sciences Inc. 5µm, 250×4.6 mm).

Extraction of Vitamin-C:

A volume of 5ml of juice were mixed with 5 ml mobile phase. The mixture was centrifuged at 5,000 xg for 5 min and filtered through PVDF Millipore filters (13 mm, 0.45 µm). Uploaded to HPLC for analysis of vitamin-C determination of AA consisted of an isocratic elution procedure with UV-Visible detection at 245 nm. Separations were carried out on a ODS-3 C18 (GL Sciences Inc. 5µm, 250×4.6 mm)., Capital HPLC, UK) fitted with a 5 mm RP C18 guard column of 20mm_4.6mm (Spherical, Optimals ODS-H, Capital HPLC, UK). The mobile phase employed was a mixture of 0.5% NaH2PO4 (pH 2.25 with H3PO4) –acetonitrile (93:7). Flow rate of the mobile phase was 1 mL/min and injection volume of 20 mL was used in quantitative analysis.

The temperature of analytical column was kept constant at 25ºC. Standard solutions and extracts were filtered through a pre filter 0.45 mm membrane before their injection into HPLC. (Shafqat *et al.,* 2012)

Chemicals and Reagents for Benzoic Acid Analysis:

Benzoic acid was obtained from Merck (Darmstadt, Germany), acetonitrile from Sigma-Aldrich Co. Ltd. (Dorset, UK) and ammonium acetates from Merck (Darmstadt Germany). All aqueous solutions were prepared using deionized water. Stock standard solution (1000 mg/L) was prepared and preserved at 2 – 8º C. The chromatographic separation was achieved using a mobile phases 40:60 acetonitrile and ammonium acetate solution. (0.4 g dissolved in 1000 ml deionized water, and adjusted at pH 4.2 with NaOH).

Chromatographic Conditions*:*

Determination of benzoic acid was performed using a Dionex HPLC system equipped with (UV/VIS) detection and a column *Luna* 5 µm C18 150 x 4.60 mm. The optimal wavelength for detection was set at 228 nm, Different samples were filtered in vacuum on a microfiltration membrane (pore size of 0.45 µm) and were placed on an ultrasonic bath for 15 minutes in order to degas before injection **(Petronela and Diacu, 2009).**

Chemicals for Benzene Analysis:

Benzene pure 99%, (Sigma-Aldrich Chemical Company), methanol HPLC grade, (Sigma-Aldrich Chemical Company)

Instrumentation and Chromatographic Conditions:

Chromatographic analysis for optimization was performed on Agilent 7890N GC with Agilent 7000 MSD (GC/MS/MS) equipped with a split/splitless injector and dynamic headspace auto-sampler. Chromatographic separations were carried out in a DB-5 ms capillary column (30 m × 0.320 mm × 0.5 µm film thickness).

The initial column oven temperature was 40 °C (1 minute); subsequently increasing 15 °C min^{-1} to 180 °C. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1} and the injector temperature was fixed at 250 °C. The quadrupole mass detector was operated in the electron impact mode at 70 eV. The ion source temperature was set at 250 °C, and the transfer line was set at 250 °C.

Analytical Procedure:

A stock solution (1 mg/mL) of benzene in methanol was prepared. From this stock solution 5, 25, 50 and 200 ppb benzene concentrations were prepared for working standard solutions using methanol as the solvent.

Samples of beverages were prepared by taking 10 ml transferred into headspace vials, and then the vials were sealed. Then the samples and standard solutions were loaded into the auto sampler for analysis. Finally, the mass spectrometer was operated in time-scheduled selected ion monitoring (SIM) mode by recording the ions (m/z): 51, 77 & 78 and the dwell time was 100 ms. The benzene peak was identified on the basis of its fragmentation pattern using the NIST Mass Spectral Search Program (NIST, Washington, D.C., USA).

Samples treatments:

Five concentrations of benzoic acid (200, 400, 600, 800 and 1000 ppm) have been added to fresh orange juice, incubated at three different temperatures (30, 40 and 50^{o} C) separately and stored for three months. Whereas, the benzoic acid concentration is limited by the FDA, as a preservative of food, to be 0.1% (FDA, 2014). Concentration1000 ppm of sorbic acid (a preservative) was added to the fresh orange juice which was considered as a control sample. The juice quantity has been divided into three parts to each degree and one part of each incubated degree has been analyzed twice for benzene content determination after one, two and three months consecutively and the mean was calculated (Techakriengkrai and Lertborwornwong, 2013).

Results and discussion:

The obtained GC/MS chromatograms for the benzene standards are shown in Fig. (1). Whereas, Fig. (2) shows the obtained MS chromatogram of the peak of benzene compound. By integrating the peak areas of the standards chromatograms, benzene standard curve was estimated. The standard curve is viewed in Fig. (3) with its linear equation and correlation coefficient.

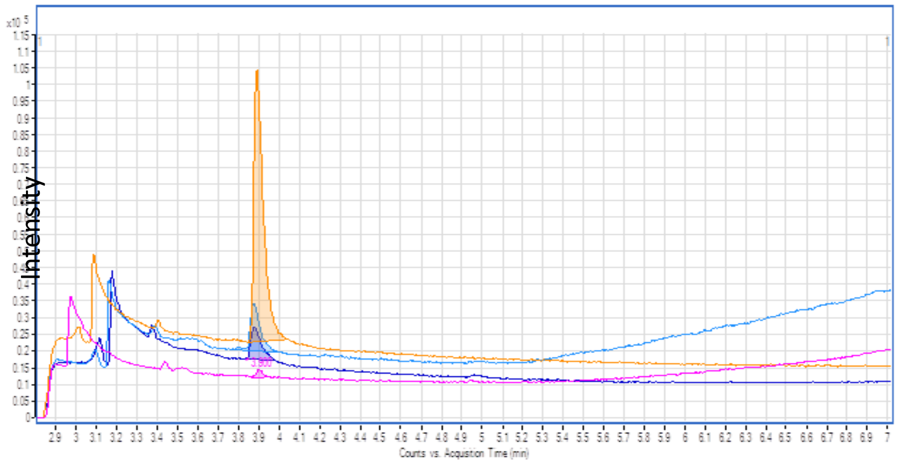


Fig. (1): GC/MC chromatograms for benzene standards

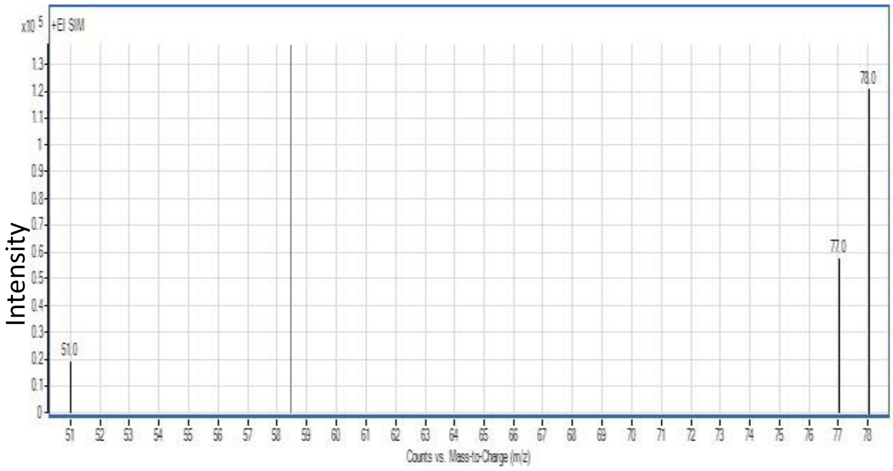


Fig. (2): The obtained MS chromatogram peak of benzene compound.

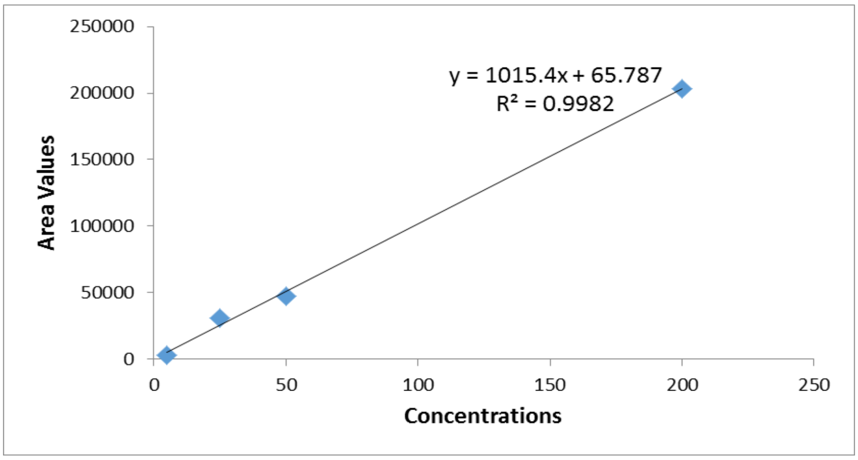


Fig. (3): The standard curve for benzene estimation

Table (1) shows the obtained results for benzoic acid, ascorbic acid and benzene contents in the chosen samples. It was noted that, benzoic acid content depends on additive preservative amount (benzoate salts) in the juice product. While, ascorbic acid content depends on the kind of the juice. The higher content of ascorbic acid was in guava juice followed by orange, strawberry and then the mango juice. Benzene content was found to be depends on both benzoic and ascorbic acid contents where the higher levels of benzene were at higher contents of both benzoic acid and ascorbic acid contents together. That suggested that benzene was produced from decarboxylation of benzoic acid in the presence of ascorbic acid as illustrated by (Lachenmeier *et al.,* 2008). The obtained results are similar with these released by (Health Canada, 2013) in its study of benzene levels in 118 beverages where the average range of benzene levels were 6.0 to 23.0 µg/L.

Table (1):Benzoic acid, ascorbic acid and benzene contents in different samples

No.	Sample	Benzoic acid content (ppm)	Ascorbic acid content (mg/100ml)	Benzene content (ppb)
1	Orange juice (trade mark 1)	224	25.2	12.6
2	Orange juice (trade mark 2)	538	22.4	17.8
3	Orange juice (trade mark 3)	245	19.6	16.8
4	Orange juice (trade mark 4)	812	20.6	20.2
5	Orange juice (trade mark 5)	448	23	15.7
6	Guava juice (trade mark 1)	208	36	16.4
7	Guava juice (trade mark 2)	511	32.2	17
8	Guava juice (trade mark 3)	229	34	14.4
9	Guava juice (trade mark 4)	788	29.9	22.7
10	Guava juice (trade mark 5)	420	37.6	21.1
11	Strawberry juice (trade mark 1)	324	12.3	13.4
12	Strawberry juice (trade mark 2)	604	11.8	12
13	Strawberry juice (trade mark 3)	236	10.6	10.9
14	Strawberry juice (trade mark 4)	830	11.2	14.7
15	Strawberry juice (trade mark 5)	389	9.9	13.2
16	Mango juice (trade mark 1)	205	5.9	8.5
17	Mango juice (trade mark 2)	581	6.6	9.1
18	Mango juice (trade mark 3)	276	5.0	7.8
19	Mango juice (trade mark 4)	788	6.1	11.8
20	Mango juice (trade mark 5)	414	6	10.7

Sodium benzoate, meanwhile, is converted into benzoic acid when placed in acidic conditions, such as a soft drink. The hydroxyl radical attacks the benzoic acid, removing the carbon dioxide and leaving benzene molecule. This reaction could take place under the same conditions prevalent in many foods and other beverages as mentioned by Patricia *et al.,* 2010. The results indicate that the levels of benzene found in soft drinks are consistent with those found in the previous study (Dominique, 2006) carried out by the Korea Food and Drug Administration (KFDA) which announced that it had detected benzene in vitamin C-enriched drinks on sale in South Korea. The detected amount of benzene ranging from 5.7 to 87.8 ppb and advised manufacturers of beverages containing more than 10 ppb of benzene to voluntarily recall their products.

The results of studying the effects of different storage temperatures (30, 40 and 50^{o}C) and different benzoic acid concentrations on benzene formation content in soft drinks are shown in tables and figures (1, 2, and 3) respectively.

Table (2): Effect of different benzoic acid concentrations on benzene contents in orange juice storing at 30ºC for a period of three months

Benzen formation / Added benzoic acid	Benzene content		
	after 1 mon. (ppb)	after 2 mon. (ppb)	after 3 mon. (ppb)
0 ppm	ND	ND	ND
200 ppm	5.4	6.9	8.8
400 ppm	6.9	8.2	9.9
600 ppm	8.8	10	11.3
800 ppm	10.2	11.9	13
1000 ppm	10.9	12.1	13.6

ND: not detected
Note: In Zero level of Benzoic Acid, Sorbic acid was added to the level of 1000 ppm

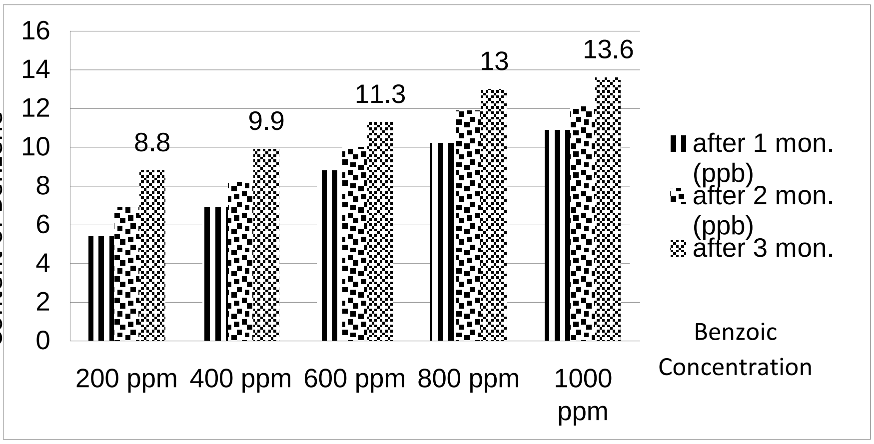
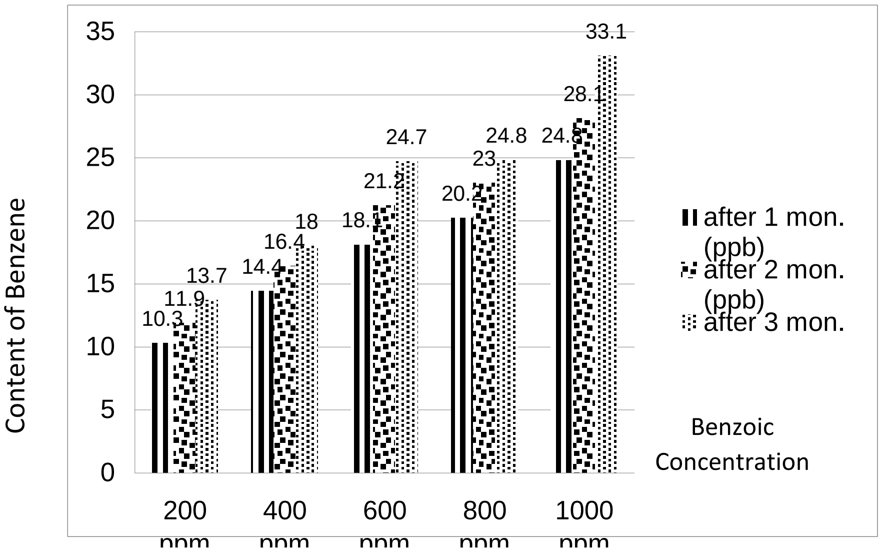


Fig (1): Benzene contents in orange juice treated with different benzoic acid concentrations storing at 30ºC

Table (3): Effect of different benzoic acid concentrations on benzene contents in orange juice storing at 40ºC for a period of three months.

Benzen formation / Added benzoic acid	Benzene content		
	after 1 mon. (ppb)	after 2 mon. (ppb)	after 3 mon. (ppb)
0 ppm	ND	ND	ND
200 ppm	10.3	11.9	13.7
400 ppm	14.4	16.4	18
600 ppm	18.1	21.2	24.7
800 ppm	20.2	23	24.8
1000 ppm	24.8	28.1	33.1

ND : not detected
Note: In Zero level of Benzoic Acid, Sorbic acid was added to the level of 1000 ppm



Fig(2): Benzene contents in orange juice treated with different benzoic acid concentrations storing at 40ºC

Table(4):Effect of different benzoic acid concentrations on benzene contents in orange juice storing at 50ºCfor a period of three months.

Benzen formation / Added benzoic acid	Benzene content		
	after 1 mon. (ppb)	after 2 mon. (ppb)	after 3 mon. (ppb)
0 ppm	ND	ND	ND
200 ppm	10.9	12.7	14
400 ppm	14.9	16.5	18.9
600 ppm	18.7	21.6	24.8
800 ppm	23.5	26.1	29.8
1000 ppm	32.4	37.3	43.2

ND : not detected
Note: In Zero level of Benzoic Acid, Sorbic acid was added to the level of 1000 ppm

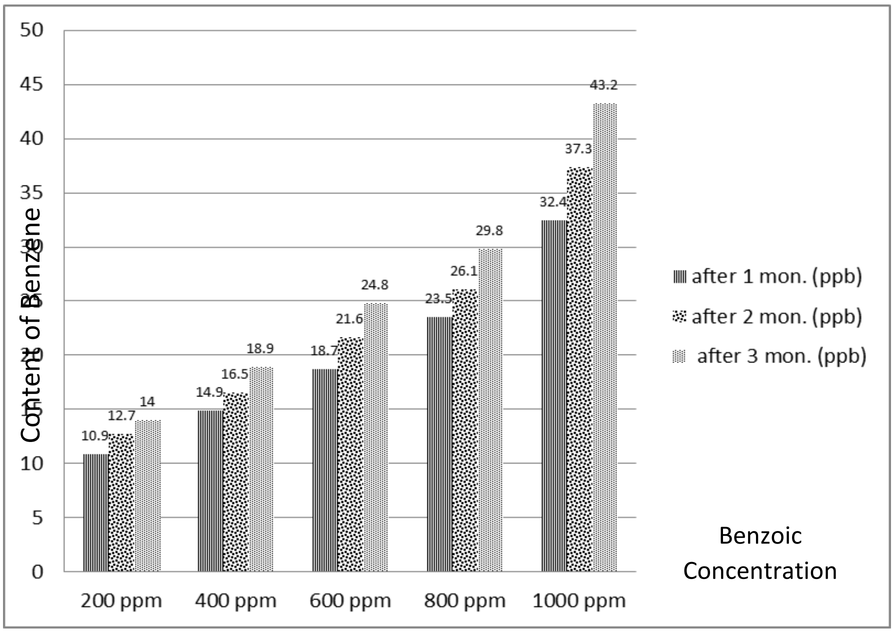


Fig (3): Benzene contents in orange juice treated with different benzoic acid concentrations storing at 50ºC.

From above results it was noticed that as the increases of a benzoic acid, the formation benzene was increased in the naturally present of ascorbic acid in juice samples. The formation of benzene was increased gradually with longer time of storage. Relative to the temperature of storage increased (30, 40 & 50ºC). Higher benzene levels were formed in these tested samples. Whereas, the highest benzene content was found at 1000 ppm concentration of added benzoic acid and at 50 ^{o}C storage temperature for three months. The lowest benzene content was found at 200 ppm concentration of added benzoic acid and at 30 ^{o}C storage temperature for one month. So, the benzene formation was found to be accelerated by storage for extended periods and at elevated temperatures. On the other hand, there was no detection of benzene in samples with added ascorbic acid (vit. C) and that because of the lack of benzoic acid. It means that the decarboxylation process to form benzene didn't occur.

The main mechanisms by which antioxidants can play their antioxidant role have been proposed, the free radical removes a hydrogen atom from the antioxidant (ArOH) that itself becomes a radical (Wright et al., 2001).RR+ArOH→RH+ArO

In this mechanism, the bond dissociation energy (BDE) of the O–H bonds is an important parameter in evaluating the antioxidant action, because the weaker the OH bond the easier will be the reaction of free radical inactivation.

CONCLUSION

This research gives awareness about the presence of benzene and its levels in some soft drinks and juices samples in Egypt. To steer clear of chemicals in drinks that can mix to form benzene. Consumers should avoid products that contain both ascorbic acid (vitamin C) and either sodium benzoate or potassium benzoate. Keeping products store at low temperatures whereas the formation of benzene could accelerate when stored for extended periods and at elevated temperatures even at only 50 ^{o}C.

Manufacturers should reformulate their products to eliminate benzene formation by using alternative preservatives of benzoic acid in high vitamin C drinks by other material such ascorbic acid.

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دراسة عن تلوث بعض المشروبات بالبنزين فى الاسواق المصرية

منال عبد المطلع عطوة و محمد حسنين الجمال

المركز الاقليمى للاغذية والاعلاف- مركز البحوث الزراعية

البنزين هو أحد الملوثات التي صنفت على أنها مسرطنة للإنسان (المجموعة 1) من قبل الوكالة الدولية لبحوث السرطان ، وقد يتعرض له الانسان من البيئة المحيطة ولكن تبين أن مستويات منخفضة من البنزين يمكن ان تنتج في المشروبات التي تحتوي على بعض المواد الحافظة والمضافات الغذائية وهي أملاح البنزوات وحمض الاسكوربيك، وهي المصادر المحتملة للتلوث بالبنزين بسبب نزع الكربوكسيل من البنزوات. وتستخدم أملاح بنزوات بوصفها عامل مضاد للميكروبات في بعض منتجات المشروبات الغازية والعصائر ، وحمض الاسكوربيك قد يتواجد إما بشكل طبيعي من مكونات عصير الفواكه في المشروبات أويتم اضافته باعتباره من المواد المضافة للأغذية كمضادات للأكسدة والمدعمة غذائيا.

وهذه الدراسة تهدف

أولا: لرصد محتويات البنزين في أنواع مختلفة من عينات العصير في الأسواق المختلفة في مصر و علاقته بالمحتوي من حمض الأسكوربيك والبنزوات.

ثانيا: دراسة تأثير تركيزات مختلفة من حمض البنزويك على محتوى البنزين في المشروبات تحت تأثير درجات حرارة التخزين المختلفة (30و40و50 ْم) ولمدة مختلفة (1و2و3 شهور).

وتبين النتائج التي تم الحصول في العينات أن محتوى البنزين يعتمد على كمية المواد الحافظة المضافة (أملاح البنزوات) للمنتج في حين محتوى حمض الاسكوربيك يعتمد على نوع العصير ونسبة الاضافة وكان أعلى محتوى من الاسكوربيك في عصيرالجوافة تليهاالبرتقال والفراولة ثم عصيرالمانجو و قد تم التاكيد علي ان محتوى البنزين المتكون يعتمد على كلا من محتويات حمض البنزويك والاسكوربيك حيث اثبتت الدراسة ان المستويات الأعلى من البنزين نتجت في المشروبات التي تحتوي على محتويات عالية من كلا البنزويك وحمض الاسكوربيك معا مما يؤكد افتراض انه يتم إنتاج البنزين من نزع الكربوكسيل من حمض البنزويك في وجود حمض الاسكوربيك. وأظهرت نتائج دراسة تأثيردرجات الحرارةالمختلفة للتخزين (30، 40و 50م) وتركيزات مختلفة من حمض البنزويك على محتوى البنزين في العصير المجهز أنه كلما زاد تركيز حمض البنزويك المضاف زاد البنزين المتكون بشكل طبيعي في عينات العصير. كما ان تكون البنزين يزيد كلما طال الوقت للتخزين وزادت درجة التخزين، في حين تم الحصول على أعلى محتوى من البنزين في تركيز 1000جزء في المليون من حمض البنزويك المضاف وعند حرارة التخزين 50ºم لمدة ثلاثة أشهر في حين كان أدنى محتوى البنزين في تركيز 200 جزء في المليون تركيزحمض البنزويك عند حرارة التخزين 30ºم و لنفس المدة(3 شهور). من ناحية أخرى لم يكن هناك تكون للبنزين في العينات المضاف لها حمض الاسكوربيك كمادة حافظة وذلك لعدم وجود حمض البنزويك والذي يعني أن عملية نزع الكربوكسيل لتكوون البنزين لم تتم.

و يمكن القول بأن البحث يعطي فكرة حول وجود البنزين ومستوياته في بعض المشروبات والعصائر في مصر. وينبغي للمستهلكين تجنب المنتجات التي تحتوي على حمض الاسكوربيك (فيتامين C) و بنزوات الصوديوم أو بنزوات البوتاسيوم. كما يجب ان يتم تخزين المنتجات في درجات حرارة منخفضة . يجب على المصنعين إعادة صياغة منتجاتهم للقضاء على تكون البنزين عن طريق استخدام مواد حافظة بديلة للحمض البنزويك -في حالة ارتفاع فيتامين C في المشروبات - مثل حمض الاسكوربيك.