

The analysis of benzene contaminant in Thai commercial non-alcoholic beverages by Headspace Gas Chromatography Mass Spectrometry

*Techakriengkrai, I. and Lertborwornwong, C.

Department of Food Technology, Faculty of Science, Ramkhamhaeng University,
KM Building, Huamark, Bangkok, 10240, Thailand

Article history

Received: 17 May 2012
Received in revised form:
2 February 2013
Accepted: 10 February 2013

Abstract

Forty eight (48) Thai commercial non-alcoholic beverages were collected nationwide. Benzoic acid and ascorbic acid were quantified employing HPLC-UV analysis, whereas benzene was quantified using headspace gas chromatography mass spectrometry (HS-GC/MS) analysis. Benzene was found in 13 products (27.08%) varying from 5.47 to 16.91 mg/L, whereas 4 samples (8.33%) were above international regulation limit (more than 10 mg/L). After re-collection of water sources and final products of these 15 products, results were found as not detected after treatment of raw water and final product at day 0. However, benzene was found significantly after kept at 30°C for 30 days. Pearson's correlation coefficient between each parameter to benzene was not significant at 95% confidence and PLS1 shows very low regression coefficient among these parameters. This work had found that the formation of benzene in samples was not resulted from their water origin, but was formed by other ingredients, which were catalyzed by storage time and conditions.

Keywords

Benzene
Benzoic acid
Ascorbic acid
Non-alcoholic beverage
Storage conditions
HS-GC/MS

© All Rights Reserved

Introduction

Benzene is considered as a human carcinogen (Andersen *et al.*, 2000; IARC, 1987) that normally exposed to human from environment (Andersen *et al.*, 2000). However, several studies reported the presence of benzene in foods and drink such as in infant foods (Fabietti *et al.*, 2004; Lachenmeier *et al.*, 2010), soft drinks (Fabietti *et al.*, 2001; Fleming-Jones and Smith, 2003; Lachenmeier *et al.*, 2010), and other solid foods (Fleming-Jones and Smith, 2003). WHO has set the guideline for a maximum content of benzene in drinking water at 10 mg/L (WHO, 1996), whereas European Union has set it at 1 mg/L (Lachenmeier *et al.*, 2008). In Thailand, however, no maximum level of benzene in drinking water or beverages has been set so far.

Analysis of benzene in food matrices had been performed by many techniques such as blender purge and trap GC/MS (Barshick *et al.*, 1995), solid-phase microextraction-GC (Huang *et al.*, 1997), headspace-mass spectrometry (Pena *et al.*, 2004), proton transfer reaction-mass spectrometry (Aprea *et al.*, 2008), and headspace-GC/MS (Vinci *et al.*, 2010). These methods provide adequate quantitative and qualitative analysis of benzene. For sensory analysis, although benzene has its own characteristic odour, its odour threshold in water is 10 mg/L (Verschueren, 1983) which is too high comparing to the concentration reported in the

literatures (Fabietti *et al.*, 2001; Fleming-Jones and Smith, 2003; Lachenmeier *et al.*, 2010). Therefore, benzene contents in foods and drinks cannot be detected in any sensory evaluation method.

The formation of benzene in beverages is not clearly understood. This can be contaminant from environment (Ezquerro *et al.*, 2004; Guo *et al.*, 2004; Liu *et al.*, 2005). However, in food industry, many studies had been focused on the reaction between benzoic acid and ascorbic acid (Aprea *et al.*, 2008; Vinci *et al.*, 2010; Lindquist and Yang, 2011) which is catalyzed by thermal processing (Lindquist and Yang, 2011), storage time and temperature (Aprea *et al.*, 2008), sunlight (Aprea *et al.*, 2008), and product's pH (Vinci *et al.*, 2010). However, most of these works had been done in the cooler climate comparing to Thailand, which has average warehouse temperature around 28°C (Thai Meteorological Department, 2009).

The purpose of this study was to determine benzene congener in commercial non-alcoholic beverages produced in Thailand using HS-GC/MS technique. Moreover, the correlations between pH, degree brix, benzoic acid, and ascorbic acid contents to the formation of benzene were also investigated in this work using multivariate statistical analysis. Concentrations acquired from this study were used in further risk assessment from benzene contents in this product category.

*Corresponding author.

Email: ittipon@ru.ac.th

Tel: +66 2 3108407 Ext. 14; Fax: +66 2 3108407

Materials and Methods

Beverage and water samples

Forty eight (48) Thai commercial non-alcoholic beverages were collected from retails and manufactories all over Thailand within two weeks of production. Forty three samples were in polyethylene terephthalate (PET) bottles, 5 samples were in transparent glass bottles, and 1 sample was in polypropylene (PP) packaging. Samples were kept at 4°C for one month before analysis. Control of each samples was kept at 4°C for 30 days in no-light environment. Thirteen untreated and treated water and final products were collected at each manufactory and kept in PET bottles at 4°C for one night (day 0 samples), and at 30°C for 30 days (day 30 samples) before analysis.

Chemicals

Benzene (99.7%) and n-propanol (99.0%) were purchased from Merck (Germany). Benzoic acid (99.5%) was purchased from Fluka (UK). Ascorbic acid (99.5%) was purchased from Sigma-Aldrich (USA). All solvents used in this work were purchased from Carlo Erba Reagent (UK).

Quantification of benzoic acid

Benzoic acid was quantified based on our previous publication (Techakriengkrai and Surankarnkul, 2007), using high-performance liquid chromatographic (HPLC) technique (Thermo Separation Products, UK) with UV-visible detector (Thermo Separation Products, UK) at the wavelength of 235 nm. Column used was Waters SPHERISORB S100DS2, 250 x 4.6 mm (Waters, UK). This isocratic method employed (60:40, 0.01M ammonium acetate buffer: Methanol (HPLC grade, Carlo Erba Reagent, UK) as mobile phase at the rate of 1 mL/min. Total running time was 15 min for each sample with the injection volume of 20 mL.

Quantification of ascorbic acid

Ascorbic acid was quantified by high-performance liquid chromatographic technique (HPLC) (Thermo Separation Products, UK) using UV-visible detector (Thermo Separation Products, UK) at the wavelength of 214 nm. Column used was C18 reverse phase (5 µm), 150 x 4.6 mm (Waters, UK). This isocratic method employed (90:10, 20 mM Potassium Phosphate, pH 3.0: Acetonitrile (HPLC grade, Carlo Erba Reagent, UK) as mobile phase at the rate of 1 mL/min. Total running time was 20 min for each sample with the injection volume of 20 mL.

Quantification of benzene

Benzene was quantified by headspace gas chromatographic technique (Agilent Technologies, 6890N) with mass spectrometry detector (Agilent Technologies, 5973) (HS-GC/MS), using complete scanning method (SCAN MODE, 20 to 100 m/z). Headspace oven was set at 80°C, while valve and transfer line temperature were set at 100°C. Sample equilibration time was 30 min, and sample injection time was 1 min. The column used was an Agilent HP5MS 30 m x 0.25 mm, and film thickness 0.25 µm. Temperature gradients were: 60°C for 2 min, then raised at 20°C/min to 200°C, and held at 250°C for 5 min. Injection port (splitless) temperatures were 250°C. Each sample was analysed in triplicate.

Data analysis

Primary data analyses were performed using Microsoft Excel v.2002 (Microsoft Corporation, USA). Statistical analyses were performed using Minitab v.14 (Minitab Inc., USA). Principal Components Analysis (PCA) and Partial least square regression (PLS) were done using Unscrambler v.7.6 (Camo ASA, Oslo).

Results and Discussions

Benzene concentration in commercial products

Figure 1 shows the GC chromatogram of benzene in sample, spiked at 20 mg/L. Detection limit of our HS-GC/MS method on benzene content was 3 mg/L, which is a little bit higher than other publications which provided lower detection limit at 0.5 mg/L (Fabiatti *et al.*, 2001; Vinci *et al.*, 2010). However, this limit provides enough sensitivity in the work, to confirm the existence of benzene in these commercial products when comparing to the WHO maximum limit at 10 mg/L (WHO, 1996). Benzene can be quantified properly with this HS-GC/MS method. Correlation coefficients (r) of benzene, benzoic acid, and ascorbic acid standard curves were 0.95, 0.98, and 0.96 respectively.

Table 1 shows the result of the first survey of 48 samples, providing product information (sample code, flavour, storage temperature, and packaging materials), pH, °brix, benzoic acid content (mg/L), ascorbic acid content (mg/L), and finally benzene content (µg/L). The amount of benzene is shown as an average of triplicate analyses (standard deviations were below 10% in all data). From product profiles, it can be seen that benzene that was found in 13 products was distributed among all flavours, packaging, and pH in this work.

Table 1. Sample details and pH, °brix, benzoic acid (mg/L), ascorbic acid (mg/L), and benzene (µg/L) contents from the first survey

| Sample Code | Flavor | Storage Temperature (°C) | Packaging | Analytical Results | | | | |
|-------------|-------------|--------------------------|-------------------|--------------------|-------|----------------------|----------------------|----------------|
| | | | | pH | °Brix | Benzoic Acids (mg/L) | Ascorbic Acid (mg/L) | Benzene (µg/L) |
| 1 | Strawberry | 25 | PET | 3.3 | 5.0 | 132 | nd | nd |
| 2 | Strawberry | 25 | PET | 2.8 | 6.2 | 160 | nd | nd |
| 3 | Strawberry | 25 | PET | 2.9 | 13.2 | 174 | nd | nd |
| 4 | Strawberry | 25 | PET | 4.1 | 9.2 | 205 | nd | nd |
| 5 | Strawberry | 25 | PET | 3.3 | 6.8 | nd | nd | nd |
| 6 | Strawberry | 25 | PET | 3.0 | 4.6 | 204 | nd | 16.91 |
| 7 | Tamarine | 25 | PET | 2.5 | 18.4 | 317 | nd | nd |
| 8 | Tamarine | 25 | PET | 2.9 | 17.2 | nd | nd | 8.61 |
| 9 | Grape | 4 | PET | 2.9 | 14.4 | nd | nd | nd |
| 10 | Grape | 25 | PET | 3.3 | 5.0 | 145 | nd | nd |
| 11 | Grape | 25 | PET | 2.8 | 6.2 | 158 | nd | nd |
| 12 | Grape | 25 | PET | 3.2 | 12.2 | 199 | nd | nd |
| 13 | Grape | 25 | PET | 4.0 | 10.0 | 178 | nd | nd |
| 14 | Grape | 25 | PET | 3.3 | 10.0 | 202 | nd | nd |
| 15 | Grape | 25 | PET | 3.3 | 5.0 | 212 | nd | 10.92 |
| 16 | Orange | 4 | PET | 3.3 | 9.0 | 165 | nd | nd |
| 17 | Orange | 4 | PET | 3.0 | 14.0 | 154 | nd | nd |
| 18 | Orange | 25 | PET | 3.1 | 6.2 | 161 | nd | 5.47 |
| 19 | Orange | 25 | PET | 2.6 | 14.0 | 197 | nd | 9.11 |
| 20 | Orange | 25 | PET | 4.1 | 9.8 | 188 | nd | nd |
| 21 | Orange | 25 | PET | 2.8 | 4.1 | 201 | nd | nd |
| 22 | Orange | 25 | PET | 3.0 | 4.4 | 168 | nd | nd |
| 23 | Orange | 25 | PET | 3.4 | 12.4 | 164 | nd | nd |
| 24 | Orange | 25 | PET | 3.3 | 14.0 | 135 | nd | nd |
| 25 | Orange | 25 | PET | 3.1 | 6.4 | nd | nd | nd |
| 26 | Orange | 25 | PET | 3.1 | 14.2 | nd | nd | nd |
| 27 | Orange | 25 | PET | 3.1 | 4.4 | 248 | nd | nd |
| 28 | Orange | 25 | PP | 5.0 | 12.6 | 111 | nd | 5.66 |
| 29 | Orange | 25 | Transparent Glass | 2.8 | 12.2 | nd | 34 | 5.80 |
| 30 | Lime | 4 | PET | 2.6 | 14.6 | nd | nd | nd |
| 31 | Lime | 25 | PET | 2.9 | 6.4 | 159 | nd | nd |
| 32 | Lychee | 25 | PET | 2.8 | 15.0 | 196 | nd | nd |
| 33 | Lychee | 25 | PET | 4.1 | 9.6 | 178 | nd | nd |
| 34 | Lychee | 25 | PET | 3.2 | 6.0 | 133 | nd | nd |
| 35 | Lychee | 25 | PET | 2.7 | 4.2 | 114 | nd | nd |
| 36 | Lychee | 25 | PP | 4.4 | 11.4 | 121 | nd | nd |
| 37 | Guava | 25 | PET | 3.3 | 12.6 | 178 | nd | nd |
| 38 | Pineapple | 25 | PET | 2.8 | 4.0 | 113 | nd | nd |
| 39 | Pineapple | 25 | PET | 3.3 | 7.0 | 158 | nd | 9.53 |
| 40 | Aloe vera | 25 | Transparent Glass | 3.5 | 10.6 | 185 | nd | 10.22 |
| 41 | Aloe vera | 25 | Transparent Glass | 3.5 | 12.0 | 189 | nd | nd |
| 42 | Aloe vera | 25 | Transparent Glass | 3.5 | 10.0 | 206 | nd | 12.10 |
| 43 | Apple | 25 | PET | 4.0 | 9.6 | 182 | nd | nd |
| 44 | Mixed Fruit | 25 | PET | 3.7 | 11.8 | 193 | nd | 7.79 |
| 45 | Mixed Fruit | 25 | PET | 3.3 | 10.2 | 199 | 48 | 6.92 |
| 46 | Mixed Fruit | 25 | PET | 3.5 | 12.4 | 191 | 20 | nd |
| 47 | Mixed Fruit | 25 | PET | 3.2 | 8.6 | 166 | nd | 8.13 |
| 48 | Mixed Fruit | 25 | PET | 3.5 | 9.4 | 200 | nd | nd |

PET = Polyethylene terephthalate
PP = Polypropylene

Table 2. Benzene contents (µg/L) in second survey

| Sample Code | Untreated Water | Treated Water | Final Product at day 0 | Final Product at day 30 |
|-------------|-----------------|---------------|------------------------|-------------------------|
| 6 | 7.66 | nd | nd | 8.72 |
| 8 | nd | nd | nd | nd |
| 15 | 5.25 | nd | nd | 4.32 |
| 18 | nd | nd | nd | 15.16 |
| 19 | nd | nd | nd | nd |
| 28 | nd | nd | nd | 9.83 |
| 29 | nd | nd | nd | 10.07 |
| 39 | nd | nd | nd | 4.00 |
| 40 | nd | nd | nd | 3.97 |
| 42 | 12.72 | nd | nd | 7.23 |
| 44 | nd | nd | nd | nd |
| 45 | nd | nd | nd | nd |
| 47 | nd | nd | nd | 9.96 |

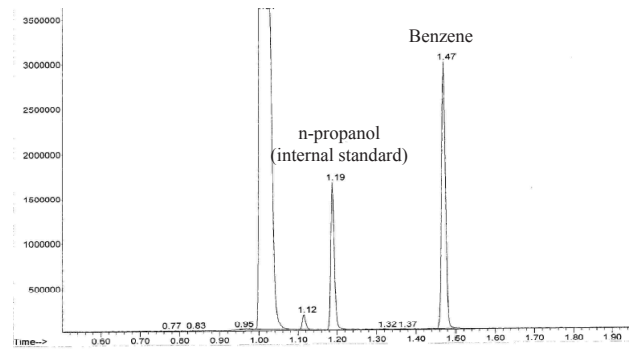


Figure 1. GC chromatogram of sample with spiked benzene (20 µg/L)

to their model, and did not correlated to benzene content.

Interestingly, 4 samples stored at 4°C were not detected in benzene. This shows similar result that was explained in model systems by Aprea *et al.* (2008) that storage temperature has direct impact on the formation of benzene from sodium benzoate and ascorbic acid. Aprea *et al.* (2008) also explained in their model systems that sugars concentration had effect on benzene formation. However, sugar concentration in this work was too low comparing

Benzene concentration in raw water, processed water, and final products at day 1 and day 30

Table 2 shows the result of the second survey of 13 products whose benzene was found in the first survey using the same product code as in table 1. From this table, benzene was first found in raw water of 3 samples (5.25, 7.66, and 12.72 µg/L respectively), which all were ground water. Then, after water treatment by reverse osmosis technique, benzene

was not detected in all processed water. Moreover, benzene was also not detected in all 13 final products at day 0 (collected after production line and kept at 4°C for one night before analysis). After being kept at 30°C for 30 days, benzene was found in 9 from 13 products (69.23%) at the concentration from 3.97 to 15.16 µg/L. This result shows clear significance of time and temperature impact on the formation of benzene compound in beverages.

Relationship between benzene, benzoic acid, and ascorbic acid content

Pearson's correlation was performed individually with pH, °brix, benzoic acid content, and ascorbic acid content, each to benzene content data in those 13 samples. Correlation between each couple was very low, and not significance (data not shown). Unlike other publications that use different level of benzoic acid in their models (Aprea *et al.*, 2008; Lindquist and Yang, 2011), our benzoic acid concentration are mostly conformed to domestic maximum level allowed (200 mg/L). Therefore, the range of benzoic acid concentration among collected samples in this survey was not systematically varied. However, 2 from 13 samples had no benzoic acid but also had 5.80 and 8.61 µg/L respectively.

Partial least squares regression (PLS1) was employed to perform the prediction of benzene content in these 13 samples using benzoic acid and ascorbic acid contents as loading. This multivariate model was performed using full cross validation method, providing very low regression coefficients (R^2) at 0.37, with high root mean squared error (RMSE) at 2.39. There was no correlation of benzene formation and the reaction of benzoic acid and ascorbic acid in this work. Therefore, the origin of benzene in storage beverage could not be concluded as a result from only benzoic acid and ascorbic acid, but also the colour substances (Gosetti *et al.*, 2005, 2007) and its packaging (Pandey and Kim, 2011) which are not stable during storage at high temperature and exposure to sunlight.

Conclusions

This work provided overall idea of benzene contents in non-alcoholic beverages sold in Thailand, especially in term of food safety. Clearly, the origin of benzene found in samples was not the processed water, but its formation from ingredients during shelf-life. There was no correlation of benzene formation and the reaction of benzoic acid and ascorbic acid. Although the benzene contents found in this study were quite low, there is a requirement for further

studies in how to control the formation of benzene during product shelf-life, especially under extreme environmental conditions such as heat and sunlight.

Acknowledgements

This work was financially supported by the Institute of Research and Development, Ramkhamhaeng University, Thailand and the Food and Drug Administration, Thailand (Thai FDA). Authors would like to thank Thai FDA for the authorization in sample collecting.

References

- Andersen, H. R., Nielsen, J. B. and Grandjean, P. 2000. Toxicologic evidence of developmental neurotoxicity of environmental chemicals. *Toxicology* 144: 121-127.
- Aprea, E., Biasioli, F., Carlin, S., Mark, T. D. and Gasperi, F. 2008. Monitoring benzene formation from benzoate in model systems by proton transfer reaction-mass spectrometry. *International Journal of Mass Spectrometry* 275: 117-121.
- Barshick, S. A., Smith, S. M., Buchana, M. V. and Guerin, M. R. 1995. Determination of benzene content in food using a novel blender purge and trap GC/MS. *Journal of Food Composition and Analysis* 8: 244-257.
- Ezquerro, O., Ortiz, G., Pons, B. and Tena, M. T. 2004. Determination of benzene, toluene, ethylbenzene and xylenes in soil by multiple headspace solid-phase microextraction. *Journal of Chromatography A* 1035: 17-22.
- Fabietti, F., Delise, M. and Bocca, P. 2001. Investigation into the benzene and toluene content of soft drinks. *Food Control* 12: 505-509.
- Fabietti, F., Ambrozzi, A., Delise, M. and Sprechini, M. R. 2004. Monitoring of the benzene and toluene contents in human milk. *Environment International* 30: 397-401.
- Fleming-Jones, M. E. and Smith, R. E. 2003. Volatile organic compounds in foods: A five year study. *Journal of Agricultural and Food Chemistry* 51: 8120-8127.
- Gosetti, F., Gianotti, V., Polati, S. and Gennaro, M. C. 2005. HPLC-MS degradation study of E110 sunset yellow FCF in commercial beverage. *Journal of Chromatography A* 1090: 107-115.
- Gosetti, F., Gianotti, V., Mazzucco, E., Polati, S. and Gennaro, M. C. 2007. Sunlight induced degradation of E133 in a commercial beverage. *Dyes and Pigments* 74: 424-432.
- Guo, H., Lee, S. C., Chan, L. Y. and Li, W. M. 2004. Risk assessment of exposure to volatile organic compounds in different indoor environments. *Environmental Research* 94: 57-66.
- Huang, S. D., Cheng, C. H. and Sung, Y. H. 1997. Determination of benzene derivatives in water by solid-phase microextraction. *Analytica Chimica Acta*

343: 101-108.

- IARC. 1987. Benzene in: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Supplement 7: (7) 1-42.
- Lachenmeier, D. W., Reusch, H., Sproll, C., Schoeberl, K. and Kuballa, T. 2008. Occurrence of benzene as heat-induced contaminant of carrot juice for babies in a general survey of beverages. *Food Additives & Contaminants* 25: 1216-1224.
- Lachenmeier, D. W., Kuballa, T., Reusch, H., Sproll, C., Kersting, M. and Alexy, U. 2010. Benzene in infant carrot juice: Further insight into formation mechanism and risk assessment including consumption data from the DONALD study. *Food and Chemical Toxicology* 48: 291-297.
- Lindquist, E. and Yang, Yu. 2011. Degradation of benzoic acid and its derivatives in subcritical water. *Journal of Chromatography A* 1218: 2146-2152.
- Liu, J. F., Li, N., Jiang, G. B., Liu, J. M., Jonsson, J. A. and Wen, M. J. 2005. Disposable ionic liquid coating for headspace solid-phase microextraction of benzene, toluene, ethylbenzene, and xylenes in paints followed by gas chromatography-flame ionization detection. *Journal of Chromatography A* 1066: 27-32.
- Pandey, S. K. and Kim, K. H. 2011. An evaluation of volatile compounds released from containers commonly used in circulation of sports beverages. *Ecotoxicology and Environmental Safety* 74: 527-532.
- Pena, F., Cardenas, S., Gallego, M. and Valcarcel, M. 2004. Direct screening of olive oil samples for residual benzene hydrocarbon compounds by headspace-mass spectrometry. *Analytica Chimica Acta* 526: 77-82.
- Techakriengkrai, I. and Surakarnkul, R. 2007. Analysis of benzoic acid and sorbic acid in Thai rice wines and distillates by solid-phase sorbent extraction and high-performance liquid chromatography. *Journal of Food Composition and Analysis* 20: 220-225.
- Thai Meteorological Department, 2009. Yearly Summary on Weather. Meteorological Department: Bangkok Thailand.
- Verschueren, K. 1983. Handbook of environmental data on organic chemicals. New York, NY, USA: Van Nostrand Reinhold.
- Vinci R. M., Canfyn, M., De Meulenaer, B., de Schaetzen, T., Van Overmeire, I. V., De Beer, J. and Van Loco, J. 2010. Determination of benzene in different food matrices by distillation and isotope dilution HS-GC/MS. *Analytica Chimica Acta* 672: 124-129.
- WHO. 1996. Guidelines for drinking-water quality. 2nd ed. Vol.2. Health criteria and other supporting information. World Health Organization, Geneva.