การศึกษาสภาวะการเกิดสารก่อมะเร็งเบนซีนในน้ำส้มพาสเจอไรส์ 25 % ที่มีกรดเบนโซอิกและกรดแอสคอร์บิกเป็นส่วนผสม

Study on Carcinogenicity Conditions of Benzene in 25 % Pasteurized Orange Juice Containing Benzoic and Ascorbic Acid as an Ingredient

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาความเป็นไปได้ในการเกิดเบนซีนที่สภาวะการผลิตและการเก็บรักษาแตกต่างกันของน้ำส้มพาสเจอไรส์ 25% ซึ่งได้แก่ ปริมาณโซเดียมเบนโซเอต และกรดแอสคอร์บิกทั้งชนิดเดี่ยวและแบบผสม ค่าพีเอช อุณหภูมิและระยะเวลาการให้ความร้อน สภาวะการเก็บรักษา (อุณหภูมิ เวลา และแสงสว่าง) โลหะหนัก และ สารจับโลหะหนัก ผลการวิจัยพบว่าการเติมโซเดียมเบนโซเอต และ กรดเบนโซอิก 200 ppm และ 120 ppm ตามลำดับ ในผลิตภัณฑ์ที่มี pH 3.7 ฆ่าเชื้อที่อุณหภูมิ 100 °ซ นาน 10 นาที เก็บรักษาที่อุณหภูมิ 30 °ซ นาน 24 ชั่วโมง ตรวจไม่พบเบนซีน แต่พบเบนซีนปริมาณสูงสุดในผลิตภัณฑ์ที่มี pH เท่ากับ 2 ปริมาณการพบเบนซีนในผลิตภัณฑ์ เพิ่มขึ้นจาก 0 เป็น 5.6 ppb ตามค่า pH ที่ศึกษาระหว่าง 3.7 - 2 ตามลำดับ การเพิ่มอุณหภูมิพาสเจอไรส์จาก 90 - 100 °ซ นาน 20 นาทีพบเบนซีน เพิ่มขึ้นจาก 0 - 8.82 ppb ตามลำดับ การเพิ่มระยะเวลาการพาสเจอไรส์ (45 °ซ) เร่งการเกิดเบนซีน มากกว่าระยะเวลาเก็บ แสงสว่างไม่มีผลต่อปริมาณเบนซีน สารโซเดียมเบนโซเอตร่วมกับกรดแอสคอร์บิกเร่งการเกิดเบนซีนมากกว่าสารเพียงชนิดเดียว (ที่อุณหภูมิ 45 °ซ) การเติมสารจับไอออนโลหะ (Disodium EDTA และ Sodium hexametaphosphate, SHMP) สามารถช่วยลดการเกิดเบนซีนได้

คำสำคัญ: กรดแอสคอร์บิก เบนซีน กรดเบนโซอิก น้ำส้ม

ABSTRACT

The objective of this experiment was to study the possibility of benzene formation at different conditions used in 25% pasteurized orange juice production and storage : benzoate and ascorbic acid alone or together, pH, heating temperature and time, storage conditions (temperature, time and light exposure), metal ions, and chelating agents. An addition of 200 ppm sodium benzoate and 120 ppm ascorbic acid to 25% orange juice, pH 3.7, pasteurized at 100 ^{O}C 10 min, stored at 30 ^{O}C 24 hrs., did not induce benzene formation. The amount of benzene increased from 0 - 5.6 ppb at pH 3.7 - 2. Increased pasteurization temperature from 90 ^{O}C to 100 ^{O}C for 20 minutes, found benzene increased from 0 to 8.82 ppb and processing time from 10 to 20 minutes at 100 ^{O}C, found benzene risen from 5.32 to 8.82 ppb. Higher storage temperature (45 ^{o}C) caused more benzene formation than storage time. Light exposure did not increase

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benzene formation. Benzene formation in product added only sodium benzoate or ascorbic acid was less than sodium benzoate and ascorbic acid together (stored at 45 ^{O}C). The addition of chelating agent (disodium EDTA and sodium hexametaphosphate, SHMP) can decrease benzene formation.

Keywords: ascorbic acid, benzene, benzoic acid, orange juice

Introduction

Benzene (C_6H_6) is recognized by the IARC (International Agency for Research on Cancer) (2018) as carcinogenic to humans and cause damage to germ cells (cell mutagen). Foods or Beverages containing high levels of benzene can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid or irregular heartbeat, death. Long-term exposure to benzene cause harmful effects on the bone marrow, resulting in anemia and excessive bleeding. It can also affect the immune system, increasing the chance of infection. Some women who breathed high levels of benzene for many months had irregular menstrual periods and a decrease in the size of their ovaries (ATSDR, 2018). Its toxicological aspects, occurrence, formation mechanisms, mitigation measures and analyzes data reporting benzene levels in foods has been review by Dos Santos et al. (2015). No limits for benzene levels in beverages and food in general studies have adopted references for drinking water in a range from 1-10 ppb. The presence of benzene has been reported in various food/beverage substances with soft drinks often reported in the literature. Although the analyses reported low levels of benzene in most of the samples studied, and some exceeded permissible limit. The available data on dietary exposure to benzene is minimal from the viewpoint of public health. Often benzene levels were low as to be considered negligible and not a health risk to the consumer, but there is still a need for more studies for a better understanding of their effects on human health through the ingestion of contaminated food. Factors that influence the formation of benzene in foods have been investigated by many scientists. McNeal et al. (1993) used aqueous models containing 0.025 % ascorbic acid and 0.04 % sodium benzoate the same concentrations used in processed beverages to study the effect of temperature and U.V. on benzene formation. A total of 300 ng / g of benzene formed in models solution stored at 45 ^{O}C or under intense U.V. light (wavelength and light intensity not reported) for 20 hours. On the other hand, only 4 ng / g of benzene formed in the dark and at room temperature. However, after 8 days, the benzene concentration had risen to 266 ng / g. Chang and Ku (1993) conducted a study similar to that of McNeal et al. (1993) using the same ascorbic acid and sodium benzoate concentration but did not add copper and iron ions to the samples. After eight days stored in the dark, the solutions had a benzene concentration of 176 ng / g. The addition of chelators, such as 0.1mM sodium EDTA and diethylene-triaminepentaacetic acid (DTPA), prevented benzene formation;100mM ethanol solutions decrease benzene formation by 90% (Chang and Ku, 1993). Nyman et al. (2010) investigated the influence of accelerated thermal conditions (40 ^{O}C or 60 ^{O}C for 24 hours or 40 ^{O}C for 15 days) and U.V. light (known wavelength and intensity for 24 hours or 7 days) on liquid models containing 0.025 % ascorbic acid and 0.04 % sodium benzoate or processed beverage samples containing one or more benzene precursors (0.04 % sodium benzoate and ascorbic acid are added to

cranberry juice). Polyethylene terephthalate (PET) bottles stabilized with Tinuvin (U.V. light filter) were used to determine how well this U.V light filter inhibits benzene formation. Benzene formation was more significant in sample heated to 40 ^{O}C. than in those exposed to U.V. or visible light. However, stabilized PET bottles reduced benzene formation in beverages containing orange juice only marginally. Among the samples not exposed to U.V. light or heat, only those with cranberry juice contained benzene (2.6 ng / g) after the storage period, possibly because benzoic acid is naturally found in cranberries. Casado et al. (2011) studied pickled green olive, cucumbers, and caper berries containing ascorbic acid and benzoic acid under three experimental conditions to determine the chemical and sensory changes induced by these additives: (0) without additives; (1) sodium benzoate; and (2) sodium benzoate and ascorbic acid. The samples were stored in plastic bottles at room temperature (20 ^{O}C – 24 ^{O}C) or refrigeration (6 ^{O}C - 9 ^{O}C). Benzene only formed in treatment 2 under storage at room temperature and plastic pouches. For refrigerated or short storage periods (<14weeks),benzene concentrations were similar to those found by McNeal et al. (1993) for pickled vegetables. Green olive stored in plastic bottles had 1.5 g / L of benzene at the end of 67-week period. Depending on the vegetable matrix and packing material, benzene concentration in samples stored for more than one year can exceed 10 g / L, which is the maximum the acceptable concentration of benzene in water

Methods

1. Effect of benzoate and ascorbic acid on benzene formation.

25% orange juice was prepared by using the same recipe as practiced in the commercial factory (water 83.5%, conc.orange juice 5%, fructose syrup 5.4%, sucrose syrup 5.8%, sodium benzoate 0.02%, citric acid 0.04 %, orange flavor 0.15 %, sunset yellow 0.005 %), pasteurized at 100 ^{o}C 10 min., cool to 70 ^{o}C then fill in PET. bottle and cool to room temperature (30 ^{o}C). Testing models consisted of sodium benzoate (0, 200 ppm) and ascorbic acid (0, 60, 120 ppm) was added to orange juice before pasteurization. Finished products were analyzed for benzene (Central Laboratory (Thailand) Co., Ltd, 2010), benzoic acid (Techakriengkrai and Surakarnkul, 2006), ascorbic acid (Kafkas et al., 2006), copper and iron (William, 2002) after storage for 24 hours. The amount of benzoate and ascorbic acid added to orange juice, which forms the highest benzene, were chosen to continue in experiment 2.

2. Effect of pH on benzene formation.

25% Orange juice was prepared by the same method as 1. The amount of sodium benzoate and ascorbic acid, which formed the highest benzene (result from 1) were added. pH of orange juice was adjusted to 2, 3, 3.7 (commercial product) and 4 with citric acid. Finished products were analyzed for benzene, benzoic acid and ascorbic acid after storage for 24 hours. pH, which formed the highest benzene, was chosen to continue in experiment 3.

3. Effect of pasteurized conditions on benzene formation.

25% Orange juice was prepared by the same method as 1. Sodium benzoate and ascorbic acid were added the same amount as 1, pH of orange juice was adjusted to the result as 2. Time and Temperature of pasteurization was varied from 100 ^{o}C, 10, and 20 min.; 90 ^{o}C, 15, 20, and 30 min. Finished products were analyzed for benzene, benzoic and ascorbic acid after storage for 24 hours. Pasteurized conditions which form the highest benzene was chosen to continue in experiment 4.

4. Effect of storage conditions on benzene formation.

Prepared pasteurized orange juice the same method as 1. Sodium benzoate 200 ppm and ascorbic acid 120 ppm were added, pH of orange juice was adjusted the same as 3, then pasteurized at Temperature and time which form the highest benzene (result from 3). Products were stored at 4 ^{O}C, 35 ^{O}C, 45 ^{O}C. with and without light exposure. Benzene were analyzed at 1, 15, 30, 45, 60, 75, 90 days of storage.

5. Effect of sodium benzoate and ascorbic acid on benzene formation in pasteurized 25 % orange juice stored at 45 ^{O}C for a different period.

Prepared pasteurized orange juice the same method as 1. Sodium benzoate 200 ppm and 120 ppm of ascorbic acid were added, pH was adjusted to 2 (result from 2), pasteurized at 100 ^{O}C 20 minutes (result from 3). Products were stored at 45 ^{o}C with light exposure (result from 4). Benzene, benzoic, and ascorbic acid were analyzed at 1, 30, 60, 90 days of storage.

6. Effect of metal ions and chelating agents on benzene formation.

Prepared pasteurized orange juice, then added with sodium benzoate and ascorbic acid, adjusted pH, pasteurized and storage at the conditions which formed the highest benzene. Chelating agent tests were disodiumethylenedia minetetraacetic acid (disodium EDTA) and sodium hexametaphosphate (SHMP), each of which varied from 75, 150, 500, 1000 ppm of orange juice. Metal ions were added in the form copper sulfate ($CuSO_4.5H_2O$) and ferrous sulfate ($FeSO_4.7H_2O$) calculated as Cu 5 ppm and Fe 15 ppm of orange juice. These two levels were the maximum allowance limits of Thai regulation (FDA, 2013). Products were stored at 45 ^{O}C with light exposure. Benzene, benzoic acid, and ascorbic acid in the products were analyzed at 1 and 30 days of storage.

7. Statistical analysis

All experiments were performed in duplicate and results are presented as mean ± S.D. Primary data and regression analyses were performed using Microsoft Office Excel (Microsoft Corp.,USA). Comparison of means was carried out using Minitab version 14 (Minitab Inc., USA) to determine significant differences ($P \leq 0.05$) between treatment tested.

Results

1. Effect of benzoate and ascorbic acid on benzene formation

Benzene was not detected in 25% pasteurized orange juice added with 200 ppm sodium benzoate and ascorbic acid 60 and 120 ppm (Table 1). The analysis result found benzoic acid 165.00 ± 2.00 - 170.00 ± 2.05 ppm in a finished product, which was in the range of 97.36 - 100.31% of original benzoic acid, means that no significant loss occurs in pasteurized juice. Benzoic acid present in the product was within Thai legal limit of beverage in a sealed container (200 ppm) (FDA, 2013). Ascorbic acid remained inpasteurized juice were 32.00 ± 3.00 - 35.00 ± 2.00 ppm from 60 ppm added; and 73.00 ± 8.00 - 81.00 ± 9.00 ppm from 120 ppm added, percent losses were 25.00 - 45.83. Copper was not detected; only iron found but not exceed the legal limit (15 ppm) (FDA, 2013).

2. Effect of pH on benzene formation

25 % pasteurized orange juice added with 200 ppm of sodium benzoate and 120 ppm of ascorbic acid, pH were adjusted to 2, 3, 3.7 and 4. pH did not change after pasteurization. Significantly ($p<0.05$) Higher benzene concentration was found at

pH 2 (5.6 ± 0.24 ppb) than pH 3 (4.40 ± 0.28 ppb) and did not detect at pH 3.7 and 4 (Table 2). It showed that pH influence the benzene formation in food. Benzoic acid in products did not change by pH, but more destruction of ascorbic acid occurred at higher pH (Table 2)

3. Effect of pasteurized conditions on benzene formation

25% pasteurized orange juice was prepared as 2, adjusted pH to 2 (highest benzene formation), then pasteurized at 100 ^{O}C for 10, 20 minutes and 90 ^{O}C for 15, 20, 30 minutes. Benzene, benzoic acid, and ascorbic acid was analyzed in the finished product. Benzene was detected only when pasteurized at 100 ^{O}C, found 5.32 ± 0.16 ppb for 10 minutes and 8.82 ± 0.22 ppb for 20 minutes, benzene formation was significantly ($P \leq 0.05$) more significant at the longer heating time and higher Temperature (Table 3). The amount of benzoic and ascorbic acid retained in pasteurized juice were not significantly different ($P \leq 0.05$) (Table 3).

4. Effect of storage conditions on benzene formation

25% pasteurized orange juice was prepared as 3, pasteurized at 100 ^{O}C for 20 minutes (highest benzene formation), then storage at 4 ^{O}C, 35 ^{O}C, 45 ^{O}C ; with and without light exposure. Benzene were analyzed every 15 days until 90 days of storage. Benzene in orange juice samples stored at 4 ^{O}C with and without light exposure showed non-significant different ($P > 0.05$) and did not change with the increase of storage time (Table 4). Benzene in the samples store at 35 and 45 ^{O}C. with and without light exposure increased significantly ($P \leq 0.05$) as storage time increased. Benzene formed in the samples stored at 45 ^{O}C was higher than at 35 ^{O}C and 4 ^{O}C respectively.

5. Effect of sodium benzoate and ascorbic acid on benzene formation in pasteurized 25% orange juice stored at 45 ^{O}C for a different period.

Benzene formation in the sample contains both benzoic, and ascorbic acid was significantly ($p \leq 0.05$) more than in the sample containing only benzoic acid or ascorbic acid. When stored at 45 ºC for 30, 60 and 90 days. (Table 5).

6. Effect of metal ions and chelating agents on benzene formation

Two chelating agents were used : disodium EDTA 75 and 150 ppm, which is the maximum allowance in multivitamin solution (https://www.fda.govfood/ingredientspackaginglabeling/foodadditivesingredients/ucm091048.htm, 2018) and SHMP 500 and 1000 ppm, which is the maximum allowance in water-based flavored drink by codex alimentarius (2017). Metal ions used were $CuSO_4.5H_2O$ and $FeSO_4.7H_2O$, calculated as copper 5 ppm and iron 15 ppm which is the maximum allowance in beverages of Thai FDA (2013). Benzene concentration in orange juice samples added with iron 15 ppm (31.67 ppb) and copper 5 ppm (35.38 ppb) was not significant different ($p > 0.05$) but significantly much more than ($p \leq 0.05$) in the sample without metal added (26.64 ppb) (table 6). The conditions without metal ions, with or without disodium EDTA and SHMP were not influenced on benzene formation at 1 day storage, but at 30 days, the amount of benzene in the sample added with disodium EDTA was higher than SHMP and without chelating agents (Table 7). Without the addition of metal ion, copper was not detected but found iron 1.87 ± 0.01 - 2.19 ± 0.07 ppm (Table 1) in orange juice sample. After storage for 30 days, the addition of SHMP 500 and 1000 ppm did not cause a significant different in

benzene formation. The same result also occurred with the addition of disodium EDTA 75 and 150 ppm, but benzene formation was significantly less when added SHMP than without chelating agent and when added disodium EDTA 75 and 150 ppm, but benzene formation was significantly less when added SHMP than without chelating agent and when added disodium EDTA respectively (table 8). Addition of copper 5 ppm, SHMP 500 and 1000 ppm did not cause benzene formed significant different, the same pattern also occurred with the addition of disodium EDTA 75 and 150 ppm, but the addition of SHMP at two concentrations caused significant less benzene formation than added disodium EDTA and without chelating agent, respectively. This finding means that disodium EDTA can chelate with copper better than iron, but SHMP is the good chelating agent for both iron and copper.

Table 1 Benzene formation, benzoic acid, ascorbic acid, Fe and Cu content in 25 % pasteurized orange juice[a] added sodium benzoate and ascorbic acid

Sodium benzoate added (ppm)	**Benzoic acid after heating (ppm)**	**Ascorbic acid added (ppm)**	**Ascorbic acid after heating (ppm)**	**Benzene (ppb)**	**Fe (ppm)[ns]**	**Cu (ppm)**
0	Not detected	0	Not detected	Not detected	1.87 ± 0.01	Not detected
200 (169.48)	168 ± 2	0	Not detected	Not detected	1.92 ± 0.02	Not detected
0	Not detected	60	32 ± 3	Not detected	1.91 ± 0.03	Not detected
0	Not detected	120	73 ± 8	Not detected	1.98 ± 0.01	Not detected
200 (169.48)	165 ± 2	60	35 ± 2	Not detected	2.04 ± 0.02	Not detected
200 (169.48)	170 ± 2	120	81 ± 9	Not detected	2.19 ± 0.07	Not detected

[a] pH 3.7, pasteurized at 100 ºC 10 min., stored at room temp for 24 hrs. before benzene analysis

Number in parenthesis calculated as benzoic acid

ns in the same column indicate non significant different ($p > 0.05$)

Table 2 Effect of pH on benzene formation, benzoic acid, ascorbic acid content in 25% pasteurized orange juice[a]

pH	Benzene (ppb)	Benzoic acid (ppm)[ns]	Ascorbic acid (ppm)
2	5.60[a]±0.24	176 ± 4	89[a] ± 10
3	4.40[b]±0.28	171 ± 6	71[b] ± 6
3.7 (commercial practiced)	Not detected	177 ± 2	65[bc] ± 6
4	Not detected	177 ± 5	52[c] ± 3

[a] added 200 ppm sodium benzoate and 120 ppm ascorbic acid, pasteurized at 100 ^{O}C 10 min., stored at room temp for 24 hrs. before benzene analysis.

ns or same letter under the same column indicate non significant different ($p>0.05$)

Table 3 Effect of processing conditions on benzene formation, benzoic and ascorbic acid content in 25% pasteurized orange juice[a]

Processing conditions	Benzene (ppb)	Benzoic acid (ppm)[ns]	Ascorbic acid (ppm)[ns]
90 ^{O}C 15 min. (commercial practiced)	Not detected	171 ± 4	80 ± 9
90 ^{O}C 20 min.	Not detected	176 ± 2	75 ± 9
90 ^{O}C 30 min.	Not detected	172 ± 3	71 ± 7
100 ^{O}C 10 min.	5.32[b] ±0.16	176 ± 2	72 ± 10
100 ^{O}C 20 min.	8.82[a] ±0.22	169 ± 2	65 ± 6

[a] added 200 ppm sodium benzoate and 120 ppm ascorbic acid, adjusted pH to 2, stored at room temperature for 24 hrs. before benzene analysis.

ns or same letter under the same column indicate non significant different ($p>0.05$)

Table 4 Effect of storage conditions on benzene formation in 25% pasteurized orange juice[a]

Storage time (day)	Benzene (ppb)					
	Stored at 4 °C		Stored at 35 °C		Stored at 45 °C	
	Dark[ns]	Light[ns]	Dark[ns]	Light[ns]	Dark[ns]	Light[ns]
1	7.31 ± 0.07	7.31 ± 0.07	7.31 ± 0.07	7.31 ± 0.07	7.31 ± 0.07	7.31 ± 0.07
15	6.13 ± 0.39	6.87 ± 0.58	10.56 ± 0.58	10.28 ± 0.37	19.46 ± 0.92	18.89 ± 0.94
30	5.95 ± 0.34	6.53 ± 0.07	12.23 ± 0.24	12.52 ± 0.48	31.89 ± 0.66	36.01 ± 2.29
45	5.53 ± 0.44	7.17 ± 0.19	14.66 ± 0.29	14.98 ± 0.50	36.16 ± 1.92	35.48 ± 3.07
60	6.82 ± 0.58	7.43 ± 0.32	17.96 ± 0.17	18.4 ± 1.47	44.31 ± 2.68	39.14 ± 0.71
75	7.29 ± 0.89	7.55 ± 0.97	24.62 ± 4.51	24.78 ± 2.83	44.54 ± 1.35	45.65 ± 2.68
90	7.23 ± 0.18	7.84 ± 0.43	29.15 ± 2.73	30.17 ± 3.70	43.16 ± 0.43	47.17 ± 3.95

[a] added 200 ppm sodium benzoate and 120 ppm ascorbic acid, adjusted pH to 2, pasteurized at 100 °C 20 min.

ns benzene concentration stored at each Temperature was non significant different ($p>0.05$) between dark and light exposure condition

Table 5 Effect of sodium benzoate and ascorbic acid on benzene formation in 25% pasteurized orange juice[a] stored at 45 °C for different period of time

Additive (ppm)		Benzene (ppb)			
Sodium benzoate	Ascorbic acid	1 day storage	30 days storage	60 days storage	90 days storage
0	0	Not detected	Not detected	Not detected	Not detected
200	0	10.35[a] ± 0.34	21.82[b] ± 1.12	37.28[b] ± 0.01	40.45[b]± 0.42
200	120	7.31[b] ± 0.07	36.01[a] ± 2.29	39.14[a] ± 0.71	47.17[a] ± 3.95
0	120	Not detected	Not detected	Not detected	Not detected

[a] pH 2, pasteurized at 100 °C 20 min.

Same letter under the same column indicate non significant different ($p>0.05$)

Table 6 Effect of metal catalysts on benzene formation in 25% pasteurized orange juice[a]

Iron (ppm)	Copper (ppm)	Benzene (ppb)	
		1 day storage[ns]	30 days storage
0	0	8.19 ± 1.67	26.64[b] ± 0.36
15	0	8.12 ± 0.39	31.67[a] ± 1.26
0	5	10.44 ± 0.24	35.38[a] ± 0.51

[a] added 200 ppm sodium benzoate and 120 ppm ascorbic acid, adjusted pH to 2, pasteurized at 100 ^{O}C 20 min. stored at 45 ^{O}C.

ns or same letter under the same column indicate no significant different (p>0.05)

Table 7 Effect of chelating agents on benzene formation in 25% pasteurized orange juice[a]

Chelating agents (ppm)		Benzene (ppb)	
Disodium EDTA	SHMP	1 day storage[ns]	30 days storage
0	0	8.19 ± 1.67	26.64[b] ± 0.36
75	0	7.04 ± 0.18	28.77[a] ± 0.25
150	0	5.84 ± 0.35	28.39[a] ± 0.25
0	500	6.71 ± 0.83	25.35[c] ± 0.21
0	1000	6.64 ± 1.26	24.77[c] ± 0.18

[a] added 200 ppm sodium benzoate and 120 ppm ascorbic acid, adjusted pH to 2, pasteurized at 100 ^{O}C 20 min. stored at 45 ^{O}C.

ns or same letter under the same column indicate no significant different (p>0.05)

Table 8 Effect of chelating agents on benzene formation in pasteurized 25% orange juice added with metal catalysts[a]

Chelating agent (ppm)		Benzene (ppb) at 1 day storage		Benzene (ppb) at 30 days storage	
Disodium EDTA	SHMP	Fe 15 ppm	Cu 5 ppm[ns]	Fe 15 ppm	Cu 5 ppm
0	0	8.12[b] ± 0.39	10.44 ± 0.24	31.67[b] ± 1.26	35.38[a] ± 0.51
75	0	12.42[a] ± 0.16	9.61 ± 0.94	38.31[a] ± 0.80	30.45[b] ± 0.35
150	0	10.76[a] ± 0.42	8.16 ± 0.5	38.82[a] ± 0.95	32.36[b] ± 1.13
0	500	7.87[b] ± 1.05	10.13 ± 0.50	25.29[c] ± 4.05	23.09[c] ± 0.15
0	1000	8.44[b] ± 0.66	7.47 ± 1.91	22.59[c] ± 0.30	22.45[c] ± 0.64

[a] added 200 ppm sodium benzoate and 120 ppm ascorbic acid, adjusted pH to 2, pasteurized at 100 ^{O}C 20 min. stored at 45 ^{O}C.

ns or same letter under the same column indicate no significant different ($p>0.05$)

Discussion

Benzene was not detected in all products consisted of sodium benzoate (0, 200 ppm) and ascorbic acid (0, 60, 120 ppm), maybe due to a short storage time (24 hours) before analysis. Sodium benzoate can breaks down into benzoic acid in acidic conditions such as fruit juice (Aprea et at., 2008); 200 ppm benzoate equivalent to 169.48 ppm benzoic acid. The analysis result found benzoic acid 165.00 ± 2.00 -170.00 ± 2.05 ppm in the finished product, which within Thai legal limit of beverage in a sealed container (200 ppm) (FDA, 2013), means that no significant loss occur in pasteurized juice which due to the heat stability of sodium benzoate (melting point at 249 ^{O}C) (Aprea et al., 2008). Losses of ascorbic acid caused by oxygen, higher pH, and heat in the pasteurization process (Cvetkovic and Jokanovic, 2009). At low acidic condition (high pH), ascorbic acid will change to unstable L-dehydroascorbic acid, then to diketogulonic acid which has no ascorbic acid activity (Lawrence, 2010). Medeiros et al. (2011) described benzene formation from the decarboxylation of benzoate to be pH - dependent and favored at acidic pH. This is why benzene formation was lower or not detected at higher pH (with a sharp drop from pH 3 - 5). When pasteurized samples at 90 ^{O}C and 100 ^{O}C, benzene was detected significantly ($P \leq 0.05$) more significant at the higher Temperature and longer heating time. It showed that more heat would accelerate the reaction to form more benzene in the product (Gardner and Lawrence, 1993; Aprea et al., 2008). The samples storage at

35 and 45 ^{O}C with and without light exposure increased significantly ($P \leq 0.05$) as storage time increased. Benzene formed in the samples stored at 45 ^{O}C was higher than at 35 ^{O}C and 4 ^{O}C respectively. The same pattern also found by Aprea et al. (2008) studied the influence of Temperature on benzene formation using aqueous models with the same ascorbic acid and sodium benzoate concentrations as those found in processed beverages. The solutions were stored at 25 ^{O}C and 45 ^{O}C for 72 hours. Benzene formation remained constant for the first 12 hours (<0.1 µg/L) but increased to 0.44 µg/L after 70 hours; in the solution stored at 45 ^{O}C, 118.5 µg/L of benzene formed after 24 hours, increased to 125 µg/L after 48 hours. In the experiment by Morsi et al. (2012), soft drink samples were incubated at 20, 45 and 90 ^{O}C for 21 days before the determination of benzene, resulted in a significantincrease in samples subjected to Temperature of 45 ^{O}C (ranging from 5.5 ppb to 7.4 ppb) and 90 ^{O}C (ranging from 25 ppb to 55.1 ppb) compared to the stored samples at 20 ^{O}C (ranging from 0.7 ppb to 1.5 ppb). Nyman et al. (2010) investigated the influence of accelerated Temperature (40 ^{O}C or 60 ^{O}C for 24 hours or 40 ^{O}C for 15 days) and U.V. light (known wavelength and intensity for 24 hours or 7 days) on liquid models containing 0.025 % ascorbic acid and 0.04 % sodium benzoate. Benzene formation was greater in samples heated to 40 ^{O}C than in those exposed to U.V. or visible light. Benzoic acid can react with the ascorbic acid in a metal catalyzed hydroxyl radical reaction (copper and iron) to form benzene (Gardner and Lawrence, 1993) or could be formed from benzoic acid change directly to benzene or change to benzaldehyde first then to benzene later (Lange et al., 2002). Disodium EDTA can react with ascorbic acid and form benzene (http://www.ehow.com -/facts5007478-whatdisodium-edta.htmal,2010). After storage for 30 days the addition of SHMP 500 and 1000 ppm and disodium EDTA 75 and 150 ppm did not cause a significant difference in benzene formation. Disodium EDTA can chelate with copper better than iron, but SHMP is an excellent chelating agent for iron and copper. This could be explained by the stability constant (log K) value of copper (18.8) is higher than iron (14.3); metal ion with higher stability constant value can form a complex with chelating agent better than metal an ion with lower stability constant value (http://www.georgeebyresearch.com/htm/stability constants.htm, 2011.,lgoe and Hui, 2001)

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