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Determination of the effect of concentration of Sodium Benzoate in Fruit Juice containing Ascorbic acid

[*1]Idongesit I. Udo and [2]Richard A. Ukpe

[1]Department of Chemical Sciences, Akwa Ibom State Polytechnic, Ikot Osurua, Akwa Ibom State,Nigeria
[2]Department of Chemistry, Federal University, Otuoke, Bayelsa State, Nigeria.

ABSTRACT

Background: This research was designed to determine the influence of the concentration ratio of sodium benzoate on the ascorbic acid (vitamin C) content of orange juice and to determine the concentration of sodium benzoate in which the reaction is effective concerning storage duration.

Methods: This was achieved by measuring the concentration of ascorbic acid used in its reaction with sodium benzoate using iodometric titration as described by AOAC 2000.

Results: It was deduced that when a high amount of sodium benzoate (preservative) was added to ascorbic acid (vitamin C) enriched fruit juice or beverages, the high concentration of this preservative caused the reduction of ascorbic acid level due to decarboxylation reaction with benzoic acid. It was also observed that it was safer to use a trace amount of sodium benzoate (between 100 to 150 mg) as observed in the reaction potency curve. This amount coincided with the prescription given by the Food and Drug Administration Agency (FDA) as the maximum allowance of sodium benzoate used as a preservative in the formulation of a beverage.

Conclusion: It was concluded that benzoic acid, an active form of sodium benzoate (preservative) when reacted with ascorbic acid in fruit juice was reduced to a potential carcinogen known as benzene. And as the reaction gradually proceeded, the ascorbic acid content diminished.

Keywords: **Ascorbic acid, Sodium benzoate preservatives, Fruit Juice, Concentrations**

1. INTRODUCTION

Food spoilage has been a common problem throughout history and much of the spoilage is caused by the activities of microorganisms or enzymatic reactions during the storage. Using chemical substances could prevent or delay food spoilage. Sodium benzoate is one of the synthetic chemical additives widely used as preservative in the food industry. Although a large number of chemical compounds are effective food preservatives, yet most of them are not allowed due to strict laws on food safety adopted by the Food and Drug Administration Agency (FDA). This is because, in-vitro, all compounds show antimicrobial effects; adding these compounds to some food products may have some health implications and as such, only a few of them are permitted for use in a food product [1, 2]. In 2011, the Research Centre for Disease Control (RCDC) in America, estimated that 128,000 people were hospitalized due to diseases transmitted through food. Nowadays, processed food makes up 75% of global societies [3, 4]. Sodium benzoate is one of the synthetic additives generally recognized as safe (GRAS). Sodium benzoate has a chemical formula $C_7H_5O_2Na$ with a molecular weight of 144.1g/mol. It is an odourless compound, soluble in water and ethanol. It is widely used as a preservative in food, pharmaceutical and cosmetic industries to prevent yeast growth and bacterial formation, sodium benzoate has an E-number of E211 [5]. It is the sodium salt of benzoic acid. According to Boris and Mandel (2011) [6], the mechanism of preservation of sodium benzoate starts with the conversion of sodium benzoate to benzoic acid, followed by the adsorption of benzoic acid into the cell of the microbes, if the intracellular pH falls to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase decreases rapidly. This reaction

* Corresponding author: Email: udoidongesit@yahoo.com:; Phone: +234 8063721278; Orcid id: 0000-0002-5563-3281

inhibits the growth and survival of microorganism that causes food spoilage. Sodium benzoate is in the category of safe additives, yet the disadvantages of synthetic preservatives such as sodium benzoate on human health have been reported to be genotoxic and neurotoxic that can cause inter-alteration in the DNA structure [7, 8]. Over the years, researchers have revealed that there is a formation of benzene in beverages and fruit juice containing sodium benzoate (as an anti-microbial agent) and ascorbic acid [9]. Literatures have shown that low levels of benzene could 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 [10]. These reactions are catalyzed by trace levels of metal ions that reduce oxygen through reactions involving hydroxyl radicals to form benzene [11]. Various arguments have been advanced on the conditions that promote the formation of this carcinogen benzene in fruit juices. According to Nyman et al., (2008) [12], a survey conducted in fruit juices to determine the presence of benzene concluded that benzene may be formed at nanogram per gram levels in some beverages containing both ascorbic acid and benzoate salts. They also reported that elevated temperature and light could stimulate these reactions, while sugar (sucrose) and ethylenediaminetetraacetic acid (EDTA) can inhibit the reactions. Zengin et al., (2010 [4], in a study found out that those preservatives such as sodium benzoate in food and drink intake could lead to low-grade inflammation in the body and this type of inflammation can be chronic in people with obesity. Animal studies have suggested that sodium benzoate can activate inflammatory pathways in the body in direct proportion to the amount consumed while benzoate preservative is responsible for some of the health challenges in life [5, 13]. According to Zhang and Ma (2013) [11], Food and Drug Administration (FDA) recommended concentration in food formulation is 0.02% for carbonated beverages, 0.05-0.1% for fruit juice and mayonnaise, 0.1% for baked foods and white layer cakes. However, literature is scanty on the concentration ratio between sodium benzoate and ascorbic content of fruit juice and beverages. Consequently, this research aimed to determine if truly there is an interaction between sodium benzoate and ascorbic acid content in fresh orange juice and also at which concentration ratio will the reaction be effective.

2. MATERIAL AND METHODS

2.1 Materials

Chemicals such as sodium thiosulphate, potassium iodide, Potassium Iodate,Starch Solution, distill water Acetic Acid, EDTA (Analytical grades) were purchased from British Drug House (London) while oranges were bought from a local farm in Uyo.

2.2 Methods

2.2.1 Sample Collection/Treatment

The orange fruits were bought from Urua Mbakara, Ikot Ekpene L.G. A. and were conveyed to the Chemistry Laboratory Akwa Ibom State Polytechnic Ikot Osurua, Ikot Ekpene . The sample were stored in a cool room for further use.The orange fruits were washed to remove dirt and pilled. The orange juice was extracted using an electric blender and the juice extract were filled into six (6) sample bottles of 1 liter capacity and various amount of sodium benzoate were added to 5 orange juice samples and labeled accordingly ranging from 100, 150, 300, 450 and 600 mg while the 6th portion of the orange juice sample was labeled as a control because sodium benzoate (preservative) was not added to it. The test samples together with the control were kept at room temperature (25℃) and the reaction was monitored daily for one week.

2.2.2 Sample Analysis

The method for analyzing or monitoring the interaction between sodium benzoate and ascorbic acid was done by applying the principle of chemical kinetics. This was done by monitoring the amount of ascorbic acid that disintegrated due to its reactions with sodium benzoate. For this to be properly estimated, the ascorbic acid content of the fresh orange juice sample was predetermined and its reactivity to decarboxylate benzoic acid which (the active form of sodium benzoate) was monitored by determining the concentration of ascorbic acid in various reacting systems containing different amount of sodium benzoate. This was done by titrating 1.0M sodium thiosulphate ($Na_2S_2O_3$) against 20 mL of each sample, 10 mL of distilled water, 10 mL of 5% potassium iodide (KI), 10 mL of 5% potassium iodate, 5 mL of acetic acid and the mixture was titration to obtain a straw colour and 3 drops of 10% starch solution was added to it and was then titrated to colourless endpoint. Then the amount of ascorbic acid present in the sample was determined as prescribed by the Association of Official Analytical Chemists (A.O.A.C, 2000) [14]. After one week of daily monitoring the reaction system, the claims for the reaction between sodium benzoate used as a

preservative and ascorbic acid (vitamin C) was duly established and the concentration ratio at which this reaction was effective and the rate for the reaction to occur were perfectly determined.

$$\text{Ascorbic acid in mg} = 2467(0.107 - 0.0136V)$$ Where V = average volume of thiosulphate consumed.

2.3 Statistical Analysis

Results were expressed as Mean ± SEM. The means werecompared using one-way analysis of variance (ANOVA). Results were considered statistically significant at $p<.05$

3. RESULTS

Table 1: shows the amount of ascorbic acid present (left) in the sample due to its reactions with sodium benzoate on the daily determination at room temperature (25 ºC).

Benzoate Added (mg)	Ascorbic acid (mg) present						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	302 ± 0.3	293 ± 0.5	282 ± 0.2	272 ± 0.4	200 ± 0.3	187± 0.2	182± 0.6
100	302 ± 0.9	299 ± 0.6	297 ± 0.5	296 ± 0.9	289 ± 0.3	281± 0.1	279± 0.9
150	302 ± 0.9	298 ± 0.5	297 ± 0.1	295 ± 0.3	290 ± 0.9	284± 0.7	282± 0.9
300	302 ± 0.4	297 ± 0.7	292 ± 0.5	286 ± 0.4	274 ± 0.5	246± 0.7	243± 0.3
450	302 ± 0.1	299 ± 0.1	298 ± 0.4	297 ± 0.1	292± 0.1	288± 0.5	285± 0.4
600	302 ± 0.5	300 ± 0.3	299 ± 0.4	298 ± 0.2	292 ± 0.4	289± 0.1	286± 0.4

Data are expressed as mean ± standard deviation SD of triplet determination.

Table 2: The table below shows the overall rate of reaction between ascorbic acid and different concentrations of sodium benzoate per day.

Amount of sodium benzoate added (mg)	Amount of ascorbic acid consumed (mg)
Control	20.0
100	3.38
150	3.33
300	9.83
450	2.83
600	2.66

4. DISCUSSION

The results obtained from this experiment presented in Tables 1 and 2 above consequently authenticate that sodium benzoate is an effective preservative since it was able to retain the appearance, flavour and colour of the test samples over the experimental period. During the experimental period, there was no observable growth of mould or fungi in the test samples except the control sample in which sodium benzoate was not added as a preservative. From the data obtained from the experiment, it can be deduced that there was a significant interaction between sodium benzoate used as a preservative and the ascorbic acid content of the fruit juice consequent upon the high reduction of the ascorbic acid content in the controlled sample (from initial concentration of 302.0 to 182.0 mg).This observation agrees with the results obtained by James, U. G. and Ukai (2011) [15]. The reaction between sodium benzoate and ascorbic acid in beverages such as fruit juice is potentially catastrophic since it produces endpoint "benzene" which is an effective carcinogen. Benzene is the product of decarboxylation of benzoic acid (the active ingredient in benzoate) in the presence of ascorbic acid which consequentially decreases the amount of ascorbic acid (vitamin C) in the fruit juice [16]. Comparatively, it could be deduced that the gradual decrease in the amount of ascorbic acid in the test samples (from day1 to day 7) as recorded in Table 1 above was a result of the denaturalization of ascorbic acid molecule in the sample due to the decarboxylation reactions that gradually took place. Figures 1-6 show the reaction curve at different concentrations of sodium benzoate. The reaction curves revealed that the decarboxylation reaction in the fruit juice gradually proceeded with time (days), was proportional to the diminishing rate of the reactant "ascorbic acid". This intrinsically explains how the reaction responded to the amount of preservative added for the experimental duration.

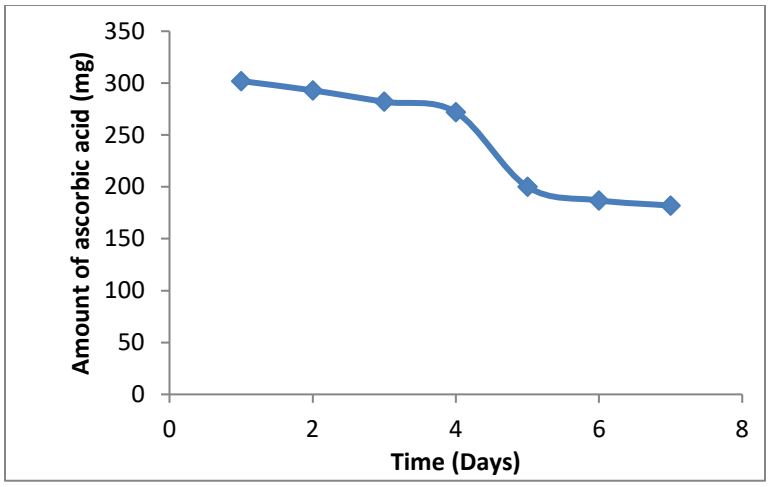

Figure 1: A graph showing the deterioration pattern of ascorbic acid in fruit juice without a preservative (sodium benzoate) for time (days)

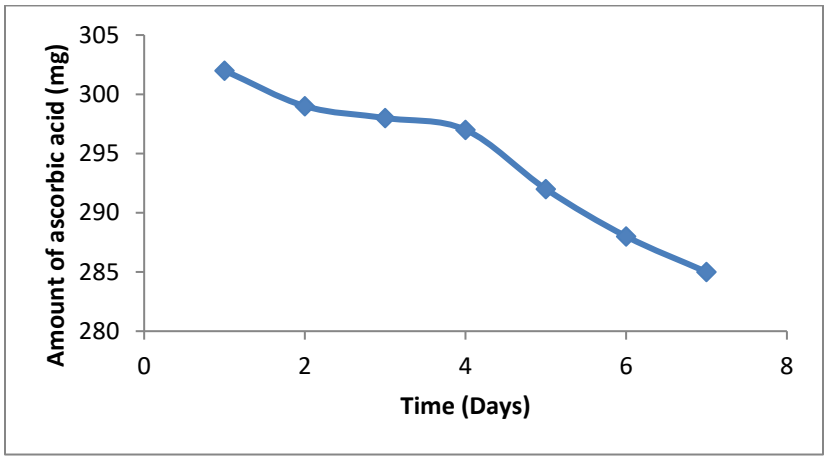

Figure 2: A graph showing the disintegration pattern of ascorbic acid using 100mg of sodium benzoate as a preservative for time (days).

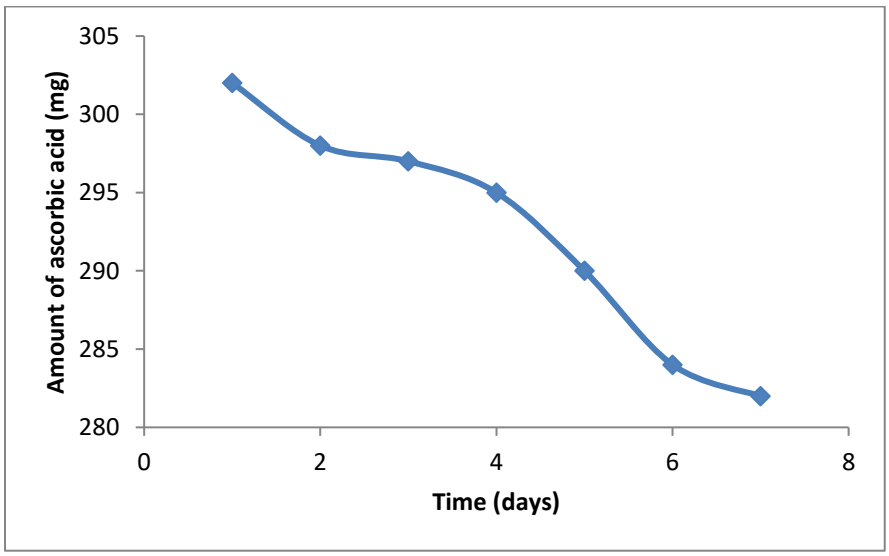

Figure 3: A graph showing the disintegration pattern of ascorbic acid using 150mg of sodium benzoate as a preservative for time (days).

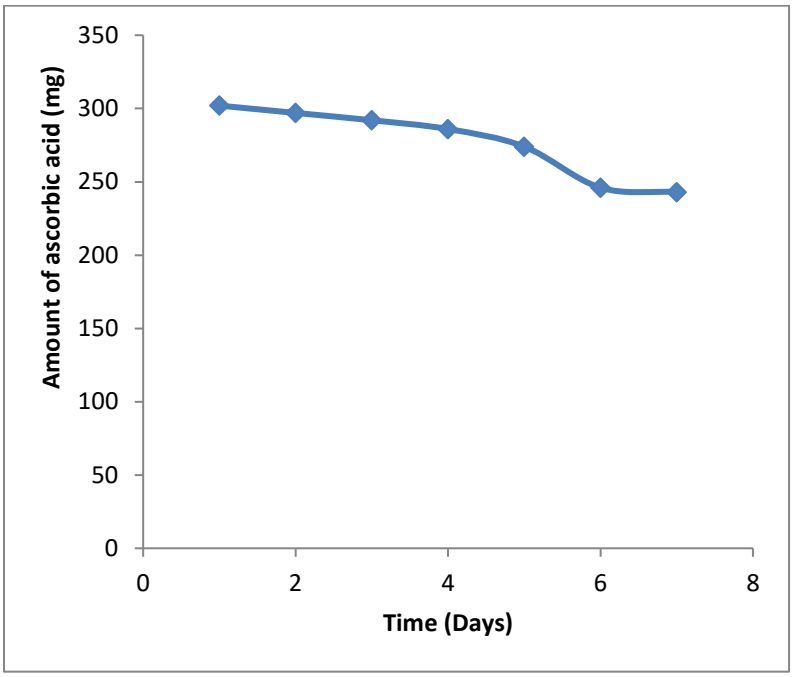

Figure 4: A graph showing the disintegration pattern of ascorbic acid using 300mg of sodium benzoate as a preservative for time (days).

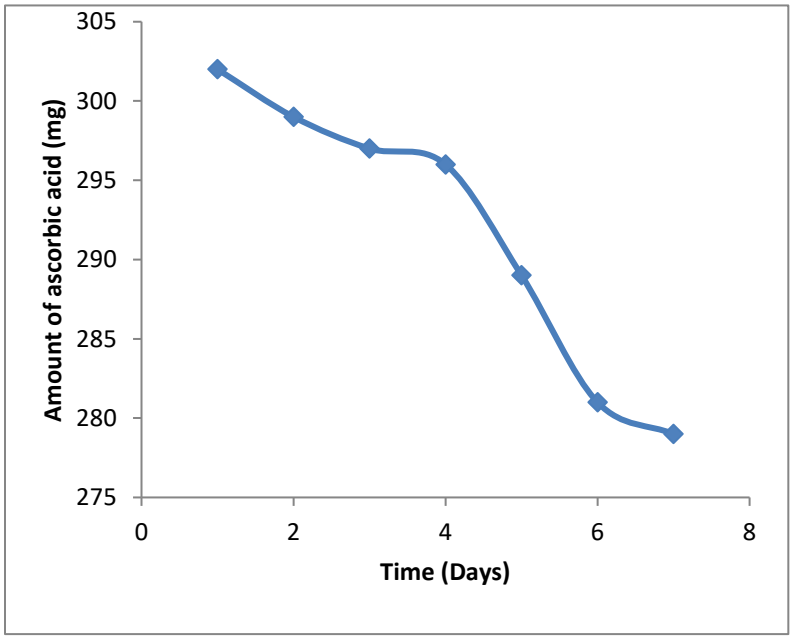

Figure 5: A graph showing the disintegration pattern of ascorbic acid using 450mg of sodium benzoate as a preservative for time (days).

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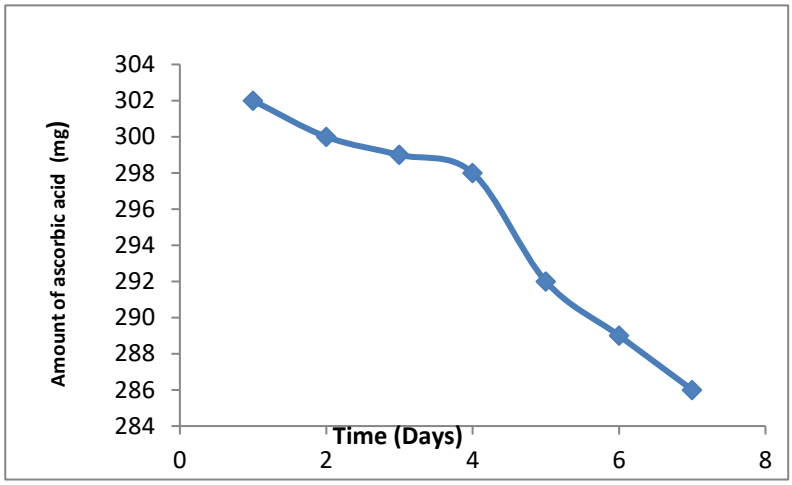

Figure 6: A graph showing the disintegration pattern of ascorbic acid using 600mg of sodium benzoate as a preservative for time (days)

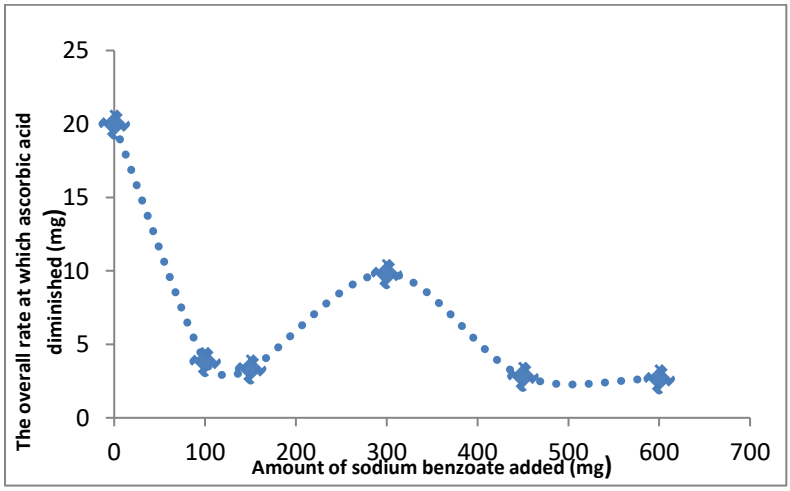

Figure 7: The figure below shows the spontaneous and the potency of the reaction for the amount of sodium benzoate added at 25 °C.

The curve above shows the effect of the concentration ratio of sodium benzoate and ascorbic acid, accelerating the decarboxylation process. As displayed in the curve above, the control sample in which the preservative was not added was highly susceptible to the denaturalization of its ascorbic acid content due to fermentation reactions and other enzymatic reactions caused by microorganisms. This, indicate that fermentation can decrease the ascorbic acid content in fruit juice. It was also observed that the potency for the reaction to proceed was insignificant in the test sample where the amount of preservative added was 100mg/ml. The reaction rate became minimal in test samples where the concentration of the preservative added was 150, 450, and 600 mg. These showed that the potency for sodium benzoate and ascorbic acid in fruit juice to react was minimal when the benzoate content in a fruit juice was small as in the case of 100 mg and 150 mg compared to the 302 mg of ascorbic acid content. The potency of the reaction becomes insignificant when the amount of preservative added was drastically higher than the ascorbic acid content of the fruit juice as observed in the test samples containing 450 mg and 600 mg of sodium benzoate respectively. The reaction potency curve in Figure 7 also shows that the rate at which sodium benzoate preservative and ascorbic acid in fruit juice reacted became alarming when there were present at similar concentration ranges as observed in the test sample containing 300 mg of the preservative.

5. CONCLUSION

It is concluded that benzoic acid, an active form of sodium benzoate (preservative) reacted with ascorbic acid in fruit juice and was reduced to a potential carcinogen known as benzene. It was also revealed that the reaction is minimal when a little or minute amount of sodium benzoate was used as a preservative. However, the rate of the reaction between these two ingredients gradually increased with time when the two ingredients were present at the same concentration ranges. But at low concentrations of sodium benzoate, the potency for the two ingredients to react readily was prevented because, under this condition, vitamin C served as a powerful free radical scavenger (antioxidant).

Concflict of Interest

The authors declare no conflict of interest.

Contribution of Authors

Initial conception ,designed, analysis and paper writing were done by Udo, I.I. while data analysis, interpretation and financing were carried out by Ukpe R. A.

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