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Analysis of saccharin and benzoic acid in regular and diet Cola-flavoured carbonated soft drinks

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Abstract
Carbonated soft drinks, especially Cola soft drinks showed a high consumption trend around the world, regardless of age, race, religion and culture. The usage of food additives plays an important role in the production of beverages to improve the quality, taste and shelf life of food products significantly. Sweeteners and chemical preservatives are some of the leading food additives to be added in soft drinks. For example, saccharin is used as the artificial sweetener and benzoic acid is widely used as preservative for soft drinks. However, the public has raised the controversial use of these food additives in food products since people are consuming soda more frequently. Therefore, the determination of the amounts of saccharin and benzoic acid in soda is essential to protect the wellbeing and safety of consumers. The aim of this study was to quantify the concentrations of saccharin and benzoic acid present in regular Cola and diet Cola that market in Malaysia, thereby assessing the compliance of the amounts of these additives with the limits set by the Malaysia Food Regulations 1985 and the Codex Food Standards. A simple and low cost procedure was applied in this study by using the spectrophotometric method for the saccharin analysis and the titrimetric method for the benzoic acid analysis individually. The results obtained indicated that the analysed regular Cola had mean concentrations of 13.89 ± 4.74 ppm and 226.6 ± 14.1 ppm for saccharin and benzoic acid respectively, whereas the analysed diet Cola had mean concentrations of 31.39 ± 2.10 ppm and 206.8 ± 28.2 ppm for saccharin and benzoic acid correspondingly. In addition, none of the analysed beverage samples was found to violate the maximum permitted levels set by the authorities. Furthermore, the independent t-test showed that there was a significant difference between the means of saccharin contents in regular Cola and diet Cola. Conversely, the independent t-test showed there was no significant difference between the means of benzoic acid contents in regular Cola and diet Cola.

Keywords: benzoic acid, carbonated soft drinks, saccharin

1. Introduction
Soft drinks, or commonly known as soda, have become one of the usual diets of modern people, regardless of age groups. Young children to elderly throughout the world love to drink soda because it is inexpensive, tastes good, helps to quench thirst, refreshing, convenient, easily accessible and people may get addicted likely due to caffeine. In addition, soft drinks become very popular among the hot temperate countries, like Malaysia. Moreover, the boosting sales of soft drinks worldwide have been contributed by the mass promotion and advertising of sodas everywhere, including in the hypermarkets, restaurants, petrol stations and even schools. Youngsters, especially young children, are usually the target group in the soda beverages industry because soft drinks that offer sweet and fizzing tastes are the most appealing to them [1].

With the advancement of food technology, food additives have been widely applicable in the beverage productions. Food additives are utilized in the food industry in order to maintain quality, texture, consistency, appearance, taste, alkalinity and acidity of food products. Thus, the large-scale production of good wholesome food and beverage at economical prices can be made possible with the usage of food additives [2]. For instance, sweeteners and preservatives are some of the major food additives added in soft drinks.

The popular regular soft drinks are typically high in sugar contents and calorie values. Consequently, excessive intake of regular soft drinks will lead to obesity and weight related health problems, including diabetes, cardiovascular disease and even cancer [3]. Hence, food manufacturers use artificial sweeteners such as saccharin and aspartame to replace regular sugars for the production of low calorie diet soft drinks. This product is recommended to prevent obesity problem [4]. Furthermore, the environmental profile of soft drinks is susceptible to microbial spoilage because of the high water activity and nutrient content in some drinks. Although the low pH in soft drinks induced by acidulants can inhibit most of the bacterial growth, they are still susceptible to yeast and mould spoilage. This is because yeast and mould can grow at pH < 3.5 and favour high sugar environment. Hence, benzoic acid and benzoates are often added as chemical preservatives to inhibit microbial spoilage, thereby extending the shelf life of soft drinks [5] [6].

Yet, consumers are more health conscious recently because soft drinks consumption have been reported to be linked with many health problems and food additives used in soft drinks have been thought to be the leading risk factor (Malaysian Endocrine and Metabolic Society 2010). Besides, there are numerous scientific studies raise the controversial use of saccharin and benzoic acid at high levels in food products. Toxicological evaluations stated that short term exposure to saccharin is associated with adverse allergic reaction while the long term exposure to saccharin is linked with weight gain, increased risks of preterm delivery and cancer [7]. On the other hand, short-term exposure to benzoic acid is associated with hypersensitivity whereas long term exposure to benzoic acid may have carcinogenic effect [8]. Therefore, the concentrations levels of saccharin and benzoic acid in soft drinks must be determined to ensure safe consumption within the permitted limits set by regulations. Moreover, cheap and rapid testing method with relative high accuracy is required for the

quality control of food additives used in food products. Objectives of this research were to determine and compare the amounts of saccharin and benzoic acid in regular and diet Cola-flavoured carbonated soft drinks available in Malaysia's supermarkets. In addition, this preliminary study also able to compare the compliance of the concentration levels of saccharin and benzoic acid in soft drinks with the Malaysia Food Regulations 1985 and the Codex General Standard for Food Additives under the Joint FAO/WHO Food Standards Programme.

2. Materials and methods

2.1 Material Studied

The samples used to investigate the amounts of saccharin and benzoic acid content were the popular Cola-flavoured carbonated soft drinks that can be easily purchased in the supermarkets in Malaysia. Two different types of Cola-flavoured carbonated canned drinks that were marketed in the shelves had been purchased, namely Regular Cola and Diet Cola. A can of Regular Cola (325 mL) is labelled with 130 kcal of energy whereas a can of Diet Cola (325 mL) is labelled with zero calories of energy.

2.2 Preparation of Saccharin Standards

A set of saccharin standard solutions were prepared by transferring 0 mL, 0.5 mL, 1 mL, 2 mL, 3 mL and 4 mL portions of saccharin standard solution (200 µg/mL) as in Table 1.0. Then, the UV-Vis spectrophotometer was used to measure the absorbance reading for each standard solution at 425 nm.

Table 1: Saccharin standard curve (Absorbance at 425 nm)

Amount of saccharin (µg)	0 (Blank)	100	200	400	600	800
200 µg/mL Standard saccharin (mL)	-	0.5	1.0	2.0	3.0	4.0
Distilled water (mL)	24.0	23.5	23.0	22.0	21.0	20.0
Nessler's reagent (mL)	1.0	1.0	1.0	1.0	1.0	1.0

2.3 Preparation of Samples

The purchased Regular Cola and Diet Cola carbonated canned drinks were first opened and degassed by undergoing water bath ultra-sonication for 24 minutes using an ultra-sonicator. This step was important to eliminate the carbon dioxide gas in carbonated soft drink samples, which can help to reduce interference of results. Next, the degassed samples would undergo clean-up method via extraction. It was an extensive sample pre-treatment step, including multiple extractions, filtration and evaporation (Food Safety and Standards Authority of India 2012). The determination of saccharin and benzoic acid content in soft drink samples have similar sample pre-treatment steps but the samples preparation were done separately for the individual analysis of different compounds.

2.4 Determination of Saccharin Content

Two mL of concentrated HCl (37%) was added to 50g of accurately weighed degassed sample in 250 mL separatory funnel and mixed. The contents of separatory funnel were extracted with 30 mL of diethyl ether for three times. In each extraction, the mixture was shaken and vented in the fume hood for several times. This was to ensure efficient separation of the two immiscible solvents. The bottom aqueous layer was poured into clean and dry 250 mL conical flask while the top ether extract was poured from the top of separatory funnel into another clean and dry 250 mL conical flask. Then, the aqueous layer was poured back into the separatory funnel for the next extraction. The separated aqueous layer and ether extract after each extraction was poured into their respective conical flasks and combined with previous extraction layer.

After three times of extraction, the combined ether extract was washed with 15 mL of distilled water by gentle swirling to remove any traces of mineral acid. Swirling of the separatory funnel was necessary to ensure distinct separation of the two solvents. After sample extraction, the analysed compound would dissolve in the top ether extract and thus the conical flask with bottom aqueous layer was discarded. Then, the conical flask with top ether extract was added with adequate anhydrous sodium sulfate to remove excess water. It was then filtered with filter paper and the solvent was evaporated on a hot plate at 50°C, measured with a thermometer. This evaporation step was carried out inside the fume hood to prevent the exposure of corrosive diethyl ether.

After the sample preparation step, the evaporated residue was digested with 6 mL of concentrated HCl (37%) and 5 mL of distilled water. It is followed by evaporation on a hot plate at 50°C inside the fume hood to 1 mL of solution with analyte and the diethyl ether was removed. This digestion step with HCl was repeated twice. This procedure allowed the hydrolysis of saccharin by HCl so that the resulting ammonium chloride can be determined by using the Nessler's reagent (AOAC 2000).

Then, the remaining cooled solution was diluted to 50 mL with distilled water in a 100 mL beaker. Two mL of this diluted solution was transferred into 25 mL volumetric flask and added with 1 mL of Nessler's reagent, then topped up to 25 mL with distilled water. Finally, the aliquot of this solution was poured into the plastic cuvette and its absorbance was measured at 425 nm using the UV-Vis spectrophotometer against reagent blank similarly prepared (Food Safety and Standards Authority of India 2012). The measurement of absorbance reading of the sample was done in triplicate for improved accuracy. Hence, the saccharin content of the sample was computed from the calibration graph. Ultimately, the mean, SD and RSD were calculated for the triplicate values of saccharin concentration in each sample.

2.5 Determination of benzoic acid content

The degassed sample accurately weighed 25g was transferred into a 250 mL separatory funnel and mixed with 10 mL of HCl (9.25%). The contents of separatory funnel were extracted with 30 mL of diethyl ether for three times. In each extraction, the mixture was shaken and vented in the fume hood for several times. This was to ensure efficient separation of the two immiscible solvents. The bottom aqueous layer was poured into a clean and dry 250 mL conical flask while the top ether extract was poured from the top of separatory funnel into another clean and dry 250 mL conical flask. Then, the aqueous layer was poured back into the separatory funnel for the next extraction.

The separated aqueous layer and ether extract after each extraction was poured into their respective conical flasks and combined with previous extraction layer.

After three times of extraction, the combined ether extract was washed with 15 mL of distilled water by gentle swirling to remove any traces of mineral acid and the conical flask with bottom aqueous layer was discarded. Conical flask with the top ether layer was passed through anhydrous sodium sulfate to remove excess water and filtered with filter paper. The solvent and its last traces were evaporated on a hot plate at 50ºC, measured with a thermometer. As a precaution, the evaporation step was carried out in the fume hood to prevent the exposure of corrosive diethyl ether.

After the sample preparation, the evaporated residue was then dissolved in 25 mL of neutralized alcohol. The neutralized alcohol was prepared fresh by adding three drops of phenolphthalein solution to a suitable quantity of ethanol and just sufficient 0.1N NaOH to produce a faint pink colour. This was to eliminate indicator neutralization error (New World Encyclopedia 2013).

A 50 mL of burette was prepared and filled with the titrant solution that was 0.05N of NaOH solution. Before the titration, air bubbles and leakage in the burette were checked, and the initial volume reading was recorded if the condition was good. Then, the analysed solution that placed inside the conical flask was ready to be titrated against 0.05N NaOH solution using phenolphthalein indicator on a white tile. The endpoint was reached when the first faint pink colour appeared for at least 30 seconds. The final volume reading of the burette was recorded to determine the volume of titrant used for each sample. The titration was repeated three times for each sample.

The titre volume obtained from the experiment was equivalent to the mixture of benzoic acid and saccharin which corresponding to the total sample. Thus, the benzoic acid content can be determined by subtracting titre of saccharin content from the titre of the total sample following the equations as presented below in (1) (Food Safety and Standards Authority of India 2012). The saccharin content in sample was first determined individually using the colorimetric procedure with Nessler's reagent as described above. The titre (B) equivalent to saccharin content of the total sample was calculated from the equation as shown in (2).

$$\text{Benzoic acid (ppm)} = \frac{(A-B)\ x\ NaOH\ x\ 122\ x\ 10^3}{W}$$

Na OH = Normality used
W = Weight of sample taken
A = Titre corresponding to total sample
B = Titre corresponding to saccharin (1)

$$B = \frac{W\ x\ S\ x\ 10^{-6}\ x\ 0.05}{N\ x\ 0.00916}\ \text{mL}$$

W= Weight of sample taken for estimation
S= Saccharin content of sample (ppm)
N= Normality of NaOH used (2)

Subsequently, the triplicate readings of benzoic acid concentration in each sample were used to calculate the mean, SD and RSD. This can help to determine the precision and repeatability of this analytical method.

3. Results

3.1 Analysis of Saccharin

Following the colorimetric procedure, a known quantity of acidified beverage sample was treated with diethyl ether to extract the saccharin and the residue was digested with HCl. Then, Nessler's reagent was used to treat the aliquot so that the absorbance of coloured product can be measured at 425 nm using the UV-Vis spectrophotometer (Food Safety and Standards Authority of India 2012). Saccharin standard are recoded as in Table 3.1. RSD is also known as the coefficient of variation (CV) because it is widely used as a universal measure of variability. Chemists extensively use the RSD (%) as the general yardstick for analysis and interpretation. Besides, the RSD (%) can be used to make comparisons across dissimilar results (Torbeck 2010) [9].

Table 3.1: Saccharin standard curves

Amount of saccharin (µg)		0 (Blank)	100	200	400	600	800
200 µg/mL standard saccharin (mL)		-	0.5	1.0	2.0	3.0	4.0
Distilled water (mL)		24.0	23.5	23.0	22.0	21.0	20.0
Nessler's reagent (mL)		1.0	1.0	1.0	1.0	1.0	1.0
Absorbance at 425 nm	1)	0	0.029	0.099	0.217	0.365	0.482
	2)	0	0.023	0.091	0.206	0.354	0.470
	3)	0	0.054	0.092	0.206	0.345	0.478
Mean absorbance ± SD		-	0.0353 ± 0.0164	0.094 ± 0.0004	0.210 ± 0.0064	0.355 ± 0.0100	0.477 ± 0.0061
RSD (%)		-	46.46	0.43	3.05	2.82	1.28

The results obtained in Table 3.1 showed that the absorbance reading at 425 nm increased as the amount of saccharin in the solutions increased. 800 µg of saccharin solution had the highest mean absorbance value which was 0.477 ± 0.0061 whereas 100 µg of saccharin solution had the lowest mean absorbance value which was 0.0353 ± 0.0164. Low readings of SD and RSD indicate high precision and repeatability of the method [9]. The SD and RSD for the mean absorbance reading of 100 µg saccharin solution were higher as compared to that of 800 µg saccharin solution. The mean absorbance reading for 100 µg saccharin solution had the highest values of SD and RSD among all the saccharin standard solutions, which were 0.0164 and 46.46% respectively. This indicated that the mean absorbance value for 100 µg saccharin solution had the lowest precision and reproducibility. This was because there might be considerable variation in the estimates of SD when the sample size of analyte was small and thus the RSD (%) was significantly high [9].

Subsequently, the calculated mean absorbance readings of the saccharin standard solutions were used to plot the saccharin standard curve of mean absorbance at 425 nm against the known amounts of saccharin. The saccharin standard curve below showed a linear graph, where the mean absorbance at 425 nm

was directly proportional to the amount of saccharin (µg) [Figure 1]. The standard curve also presented the computed equation which was y=0.0006x. Meanwhile, the R^2 value of the standard curve showed 0.9905. R^2 is a statistical measure of how close the data points are to the fitted regression line. It is also known as the coefficient of determination. The closer is the R^2 value to 1, the better the model fits the data. Hence, the R^2 value of 0.9905 indicated that the regression line fit the data almost perfectly.

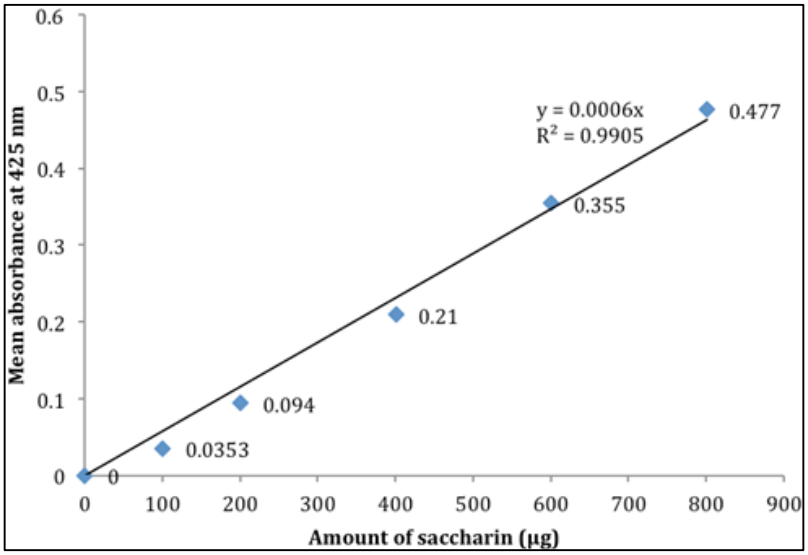

Fig 1: Standard curve of mean absorbance at 425 nm against amount of saccharin (µg).

3.2 Determination of saccharin content in samples

As a result, the amount of saccharin in Cola-flavoured soft drinks samples could be determined by substituting their respective absorbance values into the computed calibration graph which was y=0.0006x. From Table 4.2, diet Cola showed higher triplicate absorbance readings at 425 nm as compared to the regular Cola. This estimated that the diet Cola had a larger amount of saccharin content as compared to the regular Cola. From the experiment, 2 mL of diluted solution from each sample was used to react with Nessler's reagent and measure the absorbance reading at 425 nm. Thus, the calculations for the amount of saccharin in samples were based on 2 mL. Subsequently, the amount of saccharin (µg) in each sample was divided by 2 mL in order to obtain the saccharin concentration in respective sample (µg/mL) as shown in Table 3.2.

Therefore, it was proven that the saccharin concentration in the diet Cola canned drink was higher than that in the regular Cola canned drink. The diet Cola had a mean saccharin concentration of 31.39 ± 2.10 µg/mL whereas the regular Cola had a mean saccharin concentration of 13.89 ± 4.74 µg/mL [Table 3.2]. This result showed that the saccharin concentration in diet Cola was 2.3 times higher than that in regular Cola. In addition, the saccharin concentrations in both samples showed low SD which indicated this analytical method for saccharin content produced high precision and reproducibility.

However, the saccharin concentration of regular Cola had higher SD and RSD values when compared to the diet Cola. The saccharin content in regular Cola showed as high as 34.12% of RSD while the saccharin content in diet Cola only showed 6.68% of RSD. This presented that the %RSD of saccharin content in regular Cola was so much higher than that of the diet Cola and the difference between them was up to 27.44%. Hence, this indicated that the smaller the amount of saccharin content in a sample, the lower is the precision of its absorbance reading at 425 nm.

Table 3.2: Saccharin concentration in samples.

Sample		Absorbance at 425 nm	Amount of saccharin (µg)	Saccharin concentration, S (µg / 2 mL)	Mean S	SD	RSD (%)
Regular Cola	1)	0.023	38.33	19.17	13.89	4.74	34.12
	2)	0.015	25.00	12.50			
	3)	0.012	20.00	10.00			
Diet Cola	1)	0.035	58.33	29.17	31.39	2.10	6.68
	2)	0.038	63.33	31.67			
	3)	0.04	66.67	33.33			

3.3 Analysis of Benzoic Acid

The analysis of benzoic acid content in samples was based on titrimetric method. Diethyl ether was used to extract benzoic acid and saccharin from the acidified beverage samples and the mixture was then titrated against standard NaOH solution. For the determination of benzoic acid content in samples, the titre equivalent to saccharin content in each sample that was estimated separately by colorimetric assay described above was used to deduct from the total titre corresponding to benzoic acid and saccharin contents in the sample (Food Safety and Standards Authority of India 2012).

3.4 Determination of benzoic acid content in samples

All the calculations for the determination of benzoic acid content in samples were recorded in Table 3.3. Besides, the mean, SD and RSD were also recorded for each triplicate value of benzoic acid levels in samples.

Table 3.3: Benzoic acid concentration in samples.

Sample		Titre used (mL)	Benzoic acid concentration, B (ppm)	Mean B	SD	RSD (%)
Regular Cola	1)	1.00	234.75	226.6	14.1	6.2
	2)	1.00	234.75			
	3)	0.90	210.35			
Diet Cola	1)	1.00	223.10	206.8	28.2	13.6
	2)	0.80	174.30			
	3)	1.00	223.10			

The results obtained in Table 3.3 showed that the benzoic acid concentration in regular Cola was higher than that of diet Cola. This was because the regular Cola canned drink contained 226.6 ± 14.1 ppm of benzoic acid on average whereas the diet Cola canned drink contained 206.8 ± 28.2 ppm of benzoic acid on average. Yet, there was no significant difference of benzoic acid content between the two samples since their difference was only 19.8 ppm. Therefore, this result concluded that both regular and diet Cola carbonated soft drinks had similar concentration of benzoic acid.

However, the calculated SD and RSD were high for the benzoic acid levels in both of the samples as shown in Table 3.3. High values of SD and RSD indicated that this analytical method for benzoic acid content produced low precision and reproducibility. The RSD of benzoic acid content in the diet Cola showed as high as 13.6% while the RSD of benzoic acid content in the regular Cola was 6.2%. Thus, the data for benzoic concentration in regular Cola was more precise as compared to that of diet Cola.

4. Discussion

Both saccharin and benzoic acid are weak acids that partially dissociate in water with similar water solubility. This statement is supported by O'Donnell and Kearsley (2012) [10] who reported that the water solubility of saccharin was 3.4 g/L at ambient temperature while Mahindru (2009) [11] reported that the water solubility of benzoic acid was 3.44 g/L at ambient temperature. Therefore, saccharin and benzoic acid can react with a strong base, which is NaOH in the acid-base titration to produce sodium salt and water.

Limited researches have been established on the determination of saccharin levels in Cola beverages. Javeed *et al.* (2001) [12] reported the saccharin levels ranged between 46.8 ppm and 62.9 ppm in different Cola brands from the local market in Pakistan. They also established the saccharin levels within a range between 82.9 to 95.7 ppm and 93.2 to 134.1 ppm in soda beverages from orange and lemon flavoured soft drinks respectively. Besides that, there was a recent study conducted by Sik (2012) [13] who analysed 56 soft drink samples with 12 different trademarks sold in Turkey. It was found that saccharin was detected only in two out of the six analysed diet Cola products that ranged between 25.47 ppm ± 0.14 and 78.85 ppm ± 0.19. On the contrary, the regular Cola beverage was not detected with any artificial sweeteners, including saccharin, acesulfame-K and aspartame. Another research had analysed 30 samples of carbonated drinks from the Romanian market and found that the concentration of saccharin ranged between 0 and 83.75 ppm with the mean value of 9.72 ppm [14].

For comparison, the experimental values for the concentration of saccharin levels in Cola beverages were within the range established by the literature values [Table 4.1]. Yet, the saccharin content in the analysed regular Cola in this study was lower than Pakistan while studies in Turkey and Romania showed not detected (nd) for the saccharin content in certain regular Cola products. On the other hand, the saccharin content in the analysed diet Cola in this study was within the range established by Turkey and Romania studies. These studies also showed some diet Cola products having higher saccharin content than the analysed diet Cola which was only 31.39 ± 2.10 ppm.

Table 4.1: Comparison of saccharin content in samples with literature

Country	Saccharin content in regular Cola		Saccharin content in diet Cola		Reference
	Range (ppm)	Mean (ppm)	Range (ppm)	Mean (ppm)	
Pakistan	46.8 - 62.9	-	46.8 - 62.9	-	Javeed *et al.* 2001 [12]
Turkey	Nd	-	Nd - 78.85	-	Sik 2012 [13]
Romania	Nd - 83.75	9.72	Nd - 83.75	9.72	Oroian *et al.* 2013 [14]
This study	10 - 19.17	13.89 ± 4.74	29.17 - 33.33	31.39 ± 2.10	

However, from the literature, the results suggested that the amount of saccharin content in Cola beverages varied significantly from brand to brand [12, 14]. Additionally, the variability in the determination of saccharin levels in Cola flavoured soft drinks detected in these researches may be due to the differences in the production technologies in different countries. Also, the application of different analytical methods for the quantitative determination of saccharin content in soft drinks by various studies may relate with the variation in results.

Based on the Malaysia Food Regulations 1985 (amended up to 2015), saccharin is one of the permitted non-nutritive sweetening substances that may be added to low energy food. However, the Food Regulations 1985 does not establish the maximum permitted proportion of saccharin in specified food whereas acesulfame potassium and notate that may be added to the food specified have established maximum permitted levels. Yet, the Food Regulations 1985 states that the permitted non-

nutritive sweetening substance shall be written in the label on the food package whenever it is added to any food.

The Codex General Standard for Food Additives specified that the maximum permitted level of saccharin to be added as artificial sweetener into carbonated water-based flavoured drinks is 300 ppm that has been adopted in the year 2008 (GSFA 2015). The results in Table 4.2 showed that the saccharin concentration present in regular Cola and diet Cola were 13.89 ± 4.74 ppm and 31.39 ± 2.10 ppm respectively. These proved that the amounts of saccharin present in the samples were significantly below the permitted level of 300 ppm. Thus, the saccharin levels in regular and diet Cola samples were complied with the Codex standard. Furthermore, the percentage of saccharin amount used in the analysed regular Cola was only 4.63% while the percentage of saccharin amount used in the analysed diet Cola was 10.5%. Consequently, the usage of saccharin in diet Cola was proved to be higher with approximately twice the amount of saccharin used in regular Cola.

Numerous studies have conducted research on the quantitative determination of benzoic acid in soft drinks. Lino and Pena (2010) [15] had analysed 25 samples of traditional soft drinks and reported that the concentration of benzoic acid ranged between 91 ppm and 172 ppm with a mean concentration of 158 ppm. This study also determined the mean concentration of sorbic acid in soft drinks with the value of 172 ppm in a range of 78 ppm and 350 ppm. It was noted that the mean concentration of sorbic acid in traditional soft drinks was higher than that of benzoic acid for their synergistic effects on preservation. Besides that, Ree and Stoa (2011) [16] had reported 150.45 ppm of benzoic acid was found to be present in the canned diet Cola for the quantitative analysis of food additives in sugar free beverages. Moreover, the concentration of benzoic acid varied significantly between different brands of soft drink samples because Kusi and Acquaah (2014) [17] observed that the benzoic acid level ranged from not detected to an extreme of 564 ppm with the mean concentration of 70.2 ppm, in the analysis of 34 soft drinks samples in Ghana.

As for comparison, the experimental values for the determination of benzoic acid levels in Cola beverages were higher than the values reported in other researches [Table 4.2]. The mean concentrations of benzoic acid in Cola drink samples from this study were higher compared to the readings reported by studies from Portugal, Canada and Ghana as presented in Table 4.2. Hence, there was a significant difference between the experimental value and literature value of benzoic acid content in Cola-flavoured soft drinks.

Table 4.2: Comparison of benzoic acid content in samples with literature

Country	Benzoic acid content in regular Cola		Benzoic acid content in Diet Cola		Reference
	Range (ppm)	Mean (ppm)	Range (ppm)	Mean (ppm)	
Portugal	91-172 158	158	-	-	Lino and Pena 2010 [15]
Canada	-	-	-	150.4	Ree and Stoa 2011 [16]
Ghana	Nd - 564	70.2	Nd - 564	70.2	Kusi and Acquaah 2014 [17]
This study	210.35 - 234.75	226.6 ± 14.1	174.3 - 223.1	206.8 ± 28.2	

Based on the Malaysia Food Regulations 1985 (amended up to 2015), maximum permitted proportion of benzoic acid that may be added to soft drink for direct consumption is 350 ppm. Meanwhile, the international food standards, Codex General Standard for Food Additives states that the maximum permitted level of benzoates to be added into carbonated soft drinks is 600 ppm that has been adopted in the year 2004 (GSFA 2015).

The results obtained from this study showed that the benzoic acid content in regular Cola and diet Cola were 226.6 ± 14.1 ppm and 206.8 ± 28.2 ppm respectively. This indicated that the concentration levels of benzoic acid in both of the experimental samples were below the maximum permissible levels. Therefore, the concentrations of benzoic acid in both samples were complied with the Food Regulations 1985 and also the Codex standard.

Additionally, the food additives used for the production of soft drinks in Malaysia must comply with the legislation specified in the Food Regulations 1985. Since 350 ppm is the maximum permitted proportion of benzoic acid for soft drinks, the concentrations of benzoic acid in both experimental samples were just below 350 ppm. Besides, the percentage of benzoic acid amount used in regular Cola was 64.7% whereas the percentage of benzoic acid amount used in diet Cola was 59.1%. As a result, there was no significant difference for the use of the benzoic acid amount in Cola-flavoured carbonated soft drinks because the percentage difference between the two samples was only 5.6%.

However, the percentages of benzoic acid contained in both the samples were high since they were above 50%. The percentage of preservative amount used in singly or combination (sum of several percentages) must not exceed 100% based on the Food Regulations 1985. Commonly, benzoic acid is used together with other preservatives such as sorbic acid and sulfur dioxide for various food types to extend the shelf life of food products (Mahindru 2009). Since this study only analysed the concentration of benzoic acid in samples, quantitative determination of other preservatives should also be conducted to ensure compliance with the regulations and quality assurance.

5. Conclusions

Carbonated soft drinks are one of the all-time most popular beverages for consumers, especially young children and adolescents. Moreover, promotions of fast food chains are usually accompanied with carbonated soft drinks which make them more appealing and targeted to the youngsters. Nonetheless, excessive consumption of soft drinks is associated with many health concerns, including obesity, developing non-communicable diseases and even death. The usage of food additives in carbonated soft drinks has been thought to be the contributing risk factor for the developing of many health problems. Yet, the application of food additives is essential in beverages production because they can contribute significant improvement on taste and shelf life.

Sweeteners and preservatives are some of the primary food additives to be added into soft drinks. Saccharin is one of the permitted artificial sweeteners and benzoic acid is one of the permitted chemical preservatives for the production of soft drinks. However, the usage of saccharin and benzoic acid remain questionable in various food products due to the emerging health concerns presented by numerous researches. Studies showed that saccharin is associated with adverse allergic reaction during short term exposure and it is also linked with weigh gain, increased risks of preterm delivery and cancer during long term exposure. On the contrary, benzoic acid is linked with hypersensitivity and adverse carcinogenic effect for its short and long term exposures. Hence, the determination of saccharin and benzoic acid contents in soft drinks are necessary for quality control and assure safe consumption.

This research had investigated the concentration levels of saccharin and benzoic acid that present in the regular Cola and diet Cola-flavoured carbonated soft drinks using the traditional spectrophotometric assay and followed by titrimetric assay. The results showed that the regular Cola canned drink contained 13.89 ± 4.74 ppm of saccharin and 226.6 ± 14.1 ppm of benzoic acid, whereas the diet Cola canned drink contained 31.39 ± 2.10 ppm of saccharin and 206.8 ± 28.2 ppm of benzoic acid. The diet Cola had approximately twice the amount of saccharin as compared to that in the regular Cola. This was because diet Cola is a sugar-free drink, so it contained higher artificial sweetener to contribute its relative sweetness. On the other hand, the amounts of benzoic acid present in both experimental samples were similar and without significant difference between them. Furthermore, the amounts of saccharin and benzoic acid in the analysed regular Cola and diet Cola canned drinks were complied with the permissible levels established by the regulation and the international food standards.

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7. References

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