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DETERMINATION OF PRESERVATIVES AND PHYSICOCHEMICAL PROPERTIES OF FRUIT JUICE-BASED BEVERAGES

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ABSTRACT

Fruit juices and juice type beverages may have benzoates, sorbates and sulphur dioxide as preservatives. Five different categories of fruit juice-based beverages, including fruit juices, fruit nectars, fruit juice drinks, fruit drinks, and fruit cordials, were analyzed for benzoic acid, sorbic acid, and physicochemical properties such as pH, titratable acidity, degree Brix, and sugar-to-acid ratio. 15 samples were detected to contain benzoic acid while 12 samples were found to contain sorbic acid. A combination of benzoic and sorbic acids were detected in 12 samples and the remaining 36 samples did not contain any benzoic acid or sorbic acid. All the fruit juice-based beverages complied with Food Regulations 1985 for benzoic acid or sorbic acid. Brand K tropical fruit juice drink base is the only product that did not comply with the specification of CODEX standard. No violation of labelling requirement was observed in all samples. All samples tested were considered as acid food as their pH readings were below 4.6. The titratable acidity of fruit juice-based beverages ranged from 0.14 to 2.71 % (w/v). The range of Brix values measured was from 10.2 to 60.9 °Brix. Sugar-to-acid ratios calculated were ranged from 16.9 to 275.7.

1.Introduction

Fruits are perishable entities. They cannot be kept intact over long periods and tend to deteriorate. As an alternative, juices are extracted from their respective fruits to reduce these losses and add value to agricultural export (Bates *et al.,* 2001). One of the major aims of food preservation was to limit or prevent the establishment of unwanted microbial flora in food items. A variety of preservation technologies have been developed to increase the shelf-life of food goods, not only by suppressing microbial growth, but also by preserving the antioxidant capacity to meet the demands of customers (Sreerupa *et al.,* 2014). Today, the demand for these beverages increases, where the world trade has accelerated over the last decade with developing countries achieving over 60% of fruit juice exports. There are different types of fruit juice-based beverages in the market. Fruit juice is the fluid expressed from fruits by comminute, crushing, and pressing or the reconstituted product of concentrated juice and potable water (Bates *et al.,* 2001; Legal Research Board, 2010). Fruit nectar is generally made by blending a proscribed minimum percentage of fruit juices, ranging from 25 to 50 % by weight, with water and permitted sweetening substance (Bates *et al.,* 2001). On the other hand, according to Food Regulations (1985), fruit juice drink contains at least 35 % (w/v) of fruit juice, while fruit drink contains not less than 5 % (w/v) of fruit juice and fruit cordial is composed of syrup and the juice of one or more types of fruit. All fruit cordials require dilution before drinking (Legal Research Board, 2010).

Beverage manufacturing companies should ensure that every batch of their product meets consumer demand and is safe for consumption. In order to meet these quality targets, test parameters such as preservatives, pH, titratable acidity and soluble solids are used as one of the major indicators to evaluate the manufactured fruit juice products (Taylor, 2007). Sorbic acid and its derivatives are widely used to inhibit the growth of yeasts, molds, and some aerobic Gram-positive bacteria (Tucker and Featherstone, 2011). Benzoic acid and its derivatives have a similar mode of action to sorbates, but they are generally used to inhibit the growth of yeasts and molds (Taylor, 2006). Since their inhibitory effects are more effective at low pH values, they are suitable to be incorporated into fruit juice-based beverages. Chemical preservatives potassium sorbate and sodium benzoate are widely found in fruit juice and soft beverages (Magomya *et al.,* 2020). Although preservatives such as benzoic acid and sorbic acid are permitted in fruit juice beverages, the levels should not exceed the safety limits as they can be harmful to human health at high concentrations (Dong and Wang, 2006). The higher the concentration of Benzoic acid the lower the rate of growth of the microbial isolates (Oladipo *et al.,* 2010). Besides meeting quality targets, manufacturers should also ensure their product is in compliance with its respective label claim. Some unethical manufacturers tend to actively hide the actual content of fruit juices and adulterate juices with sugar, water or inferior juices (Bates *et al.,* 2001). In some cases, products labelled with "no preservatives added" may actually contain detectable number of preservatives. However, the advancements in analytical chemistry and instrumentation today make adulteration easier to detect (Nagy and Wade, 1995).

Commercially accessible fruit juices are drunk by people of all ages all over the world, and if not properly handled, this healthful drink can be harmful to human health (Ahmed *et al.,* 2018). This research aims to study the amount of benzoic and sorbic acids present in fruit juice-based beverages and to compare their amount with Food Regulations 1985 and CODEX general standard. Besides determining the amount of these preservatives in fruit juice beverages, parameters such as pH, soluble solids content and titratable acidity are evaluated in this research.

2. Materials and methods

2.1. Materials

A total of 75 samples were purchased from local supermarkets and hypermarkets in Klang Valley. In this research, fruit juices and juice beverages were classified into five categories: fruit juice, fruit nectar, fruit cordial, fruit juice drink and fruit drink. 15 samples of five varieties were chosen from each category for analysis.

2.1.1. Preparation of Samples

Ready-to-drink fruit juice beverages were centrifuged at 4500 rpm, at the temperature of 20°C for 15 minutes (Eppendort, Germany) and the supernatant diluted with 1: 10 ratio. On the other hand, fruit beverage concentrates, were reconstituted with ultrapure water (1: 100 ratio) before centrifugation.

2.2. HPLC Analysis

The HPLC analysis was performed using Agilent 1200 series HPLC system (Agilent Technologies, United States of America) equipped with vacuum degasser, G1311A Quaternary Pump, G1329A Auto-sampler, G1316A Column Thermostat, column compartment and G1315D Diode Array Detector. The chromatographic separation was done on ZORBAX Eclipse XDB-C18 analytical column (5 µm, 150 mm x 4.6 mm) at the wavelength of 235 nm. Methanol-acetate buffer (pH 4.4) were used as mobile phase (Saad *et al.,* 2005), while benzoic and sorbic acid were used a standard, with equation of 30.289x+5.1221 and 59.931x+41.833, respectively with R^2= 0.99. Each sample was analyzed for 15 minutes, with a flow rate of 1 mL per minute, and the injection volume was 10 µL.

2.3. pH and Titratable Acidity

The pH of the samples was measured using a digital pH meter (Mettler Toledo, USA) (Chang *et al.*, 2020). Titratable acidity was estimated according to Pui *et al.* (2018), where fruit juice was titrated with 0.1 M NaOH solution until the color turned from clear to pink.

2.4. Brix value and Brix-to-Acid ratio

Three drops of the juice were placed measured with refractometer (0-32 °Brix) in accordance to Pui *et al.* (2018). The Brix-to-acid ratio of fruit juice-based beverages was determined following the equation below:

Equation (1):

$$\text{Brix-to-acid Ratio} = \frac{\text{Degree Brix value } (^{\circ}Bx)}{\text{Titratable acidity (g per 100 mL)}}$$

2.5. Statistical analysis

All the data were analyzed using Minitab 17 statistical software (Minitab Inc., USA). One-way analysis of variance (ANOVA) and Tukey's HSD test was used to determine the significant differences ($p \leq 0.05$) among the fruit juice beverage samples tested. All the parameters tested in this study were determined in triplicate.

3.Results and discussions

3.1. Analysis of Benzoic Acid and Sorbic Acid in Fruit juice-based Beverages with HPLC

Table 1, figure 1 showed levels of benzoic and sorbic acids in five different categories of fruit juice-based beverages, which include fruit juice, fruit nectar, fruit juice drink, fruit drink, and fruit cordial. Among these 75 samples, 15 samples were detected to contain benzoic acid, while 12 samples were found to contain sorbic acid. A combination of benzoic and sorbic acids was detected in 12 samples, and the remaining 36 samples did not contain any benzoic acid or sorbic acid. As shown in Table 1, Brand K's tropical fruit juice drink base has the highest level of benzoic acid with the concentration of 690.0 ppm while Brand D orange drink has the highest level of sorbic acid with the concentration of 256.9 ppm. In general, fruit juice drinks have the highest average amount of benzoic acid, followed by fruit cordials, fruit drinks, and fruit juices. On the other hand, fruit drinks contained the highest average concentration of sorbic acid, followed by fruit juice drinks, fruit cordials, and then fruit juices. From the label claims Brand C pineapple juice and Brand D orange juice were purely extracted from fruits. Others were made by reconstituting concentrated juices. Brand E apple juice contained both benzoic and sorbic acids at the concentrations of 149.4 ppm and 104.7 ppm, respectively. All the tested fruit nectars complied with their label claims and did not violate Food Regulations 1985 and CODEX general standards for benzoic acid or sorbic acid. All the tested fruit juice drinks also complied with Food Regulations 1985. Brand K's tropical fruit juice drink base did not comply with the specification of the CODEX standard as its benzoic acid level has exceeded 600 ppm.

Drinks made of different fruits will give different intrinsic properties such as acidity and chemical composition. The differences in these properties will affect the type, and the number of chemical preservatives added. Here, some category of fruit juice drink has only declared the presences of permitted preservatives without indicating the type of preservative incorporated. However, both Brand K tropical fruit juice drink base and blackcurrant fruit juice drink claimed the usage of sulphur dioxide in their respective products. Since both of these beverages were found to contain benzoic acid, it could be predicted that these two products were added with more than one type of preservatives. Hence, further inspection of the products must be performed to measure other preservatives that may present. None of the fruit drinks tested in this study has exceeded the legal limits for benzoic acid or sorbic acid imposed by Food Regulations 1985. Among all the five types of fruit cordials, Table 1 presented, the highest amount of benzoic acid was detected in Brand R mixed fruit cordial at the concentration of 494.3 ppm. Brand S lychee cordial has the highest amount of sorbic acid at the concentration of 106.9 ppm. All the products in this group were by the requirement of Food Regulations for both preservatives and have complied with CODEX general standard for benzoic acid. According to fruit cordial label, another preservative known as sodium metabisulphite (E 223) was added. Thus, for preservatives besides benzoic and sorbic acids, other methods must be developed to measure and quantify other kinds of preservatives and also to determine the compliance of products with label claims and regulations.

The study indicated a preference for incorporating benzoates over sorbates into fruit juice-based beverages even though sorbates are less toxic and less obstructive in terms of taste and allergic reactions than benzoates (Taylor, 2006; World Health Organization, 2000; Tfouni and Toledo, 2002). This may be due to the lower price of benzoates and the higher solubility of benzoates (World Health Organization, 2000; Mahindru, 2008). Preservatives such as benzoic acid, sorbic acid, methyl paraben, and propyl paraben were identified and quantified concurrently in 50 different fruit juice products using a unique RP-HPLC technique that was designed, verified, and used (Islam *et al.,* 2019). Mahmoud *et al.* (2017) analysis sorbic acid and benzoic acid in different food commodities using reversed- phase high performance liquid chromatography (RP-HPLC). High-pressure liquid chromatography was used to detect sorbic acid and benzoic acid in yoghurt, tomato and pepper paste, fruit juices, chocolates, soups, and chips in Turkey (HPLC) (Cakir and Cagri-Mehmetoglu, 2014).

Brand E apple juice, Brand O tropical fruit drink base, Brand S lychee cordial, and Brand T mango cordial contained both benzoic and sorbic acids. This is because benzoic acid can act synergistically with other preservatives. The combinations of benzoic acid and sorbic acid have been reported to inhibit many bacterial strains better than either of these alone (Tucker and Featherstone, 2011; Taylor, 2006; Fellows, 2000). Ekanem and Ekanem (2018) suggest that a combination of chemical and natural preservation, as well as cooling, was ideal for the long-term preservation of apple juice. A total of 36 fruit juice beverage samples did not contain any benzoic and sorbic acids. Manufacturers today apply hurdle principles to preserve the quality of fruit juice beverages (Tucker and Featherstone, 2011; Taylor, 2006; Fellows, 2000). All the samples analyzed are low acid food. By applying pasteurization, spoilage micro-organisms can be destroyed (Bates *et al.*, 2001; Fellows, 2000). Also, for some products, for instance, the cranberry and mixed fruit juices in this study were produced by using aseptic technology. This processing, together with other barriers that combat spoilage are sufficient to destroy harmful micro-organisms, and therefore, benzoates and sorbates, can be omitted to reduce the cost. Among all the 36 samples that could not detect the presence of benzoic acid or sorbic acid, only 6 samples have claimed to contain permitted preservatives. In this case, further studies should be implemented to quantify other possible preservatives.

Even though some category of fruit juice has declared the absence of preservative on their respective label claims. However, a traceable amount of benzoic acid was detected in cranberry and mixed fruit juice because cranberries contain a natural amount of benzoic acid at approximately 150 ppm when calculated as sodium benzoate (Coppola and Starr, 1988; Pylypiw and Grether, 2000). Due to the natural occurrence of benzoic acid in cranberry juice, it was then concluded that all the fruit juice products analysed in this study did not violate the regulation of labelling. The differences in level of preservatives for the same type of product could be caused by the variation in the combined or synergistic activity of several additives, intrinsic product parameters such as composition and acidity, and extrinsic factors such as processing temperature, storage atmosphere, and temperature (Dauthy, 1995). No benzoic acid or sorbic acid was detected in all beverages categorized under the group of fruit nectar. This was because the intrinsic characteristics of these sample products, together with effective processing methods, were adequate to combat spoilage. Therefore, the usage of benzoic acid or sorbic acid as chemical preservatives could be omitted (Dauthy, 1995). Non-thermal treatment seems to be a promising and practical method for preserving fruit juice and beverages. The goods made using these processes have a number of advantages over typical thermal processing, including the preservation of sensory attributes and nutritional contents (Rupasinghe and Yu, 2012).

Food Regulations 1985 has classified fruit juice drinks, fruit drinks, and fruit cordials as soft drinks. The maximum level of benzoic acid or sorbic acid permitted by this regulation is 350 ppm for ready-to-drink soft drinks and 800 ppm for soft drinks requiring dilution. CODEX general standard has defined fruit juice drinks, fruit drinks, and fruit cordials as non-carbonated water-based flavoured drinks. The maximum level of benzoic acid allowed by CODEX commodity committees is 600 ppm. However, this standard did not specify the maximum allowable concentration of sorbates in these non-carbonated water-based flavoured drinks. This is because sorbates are less toxic than benzoates (Taylor, 2006). Sorbates are generally considered to be among the safest food preservatives in use, and therefore, only Acceptable Daily Intake (ADI) of sorbic acid was estimated at 25 mg/kg body weight (Taylor, 2006; Wood *et al.,* 2004).

As per the FAO/WHO Expert Panel on Food Additives, daily intakes of benzoic acid and sorbic acid should be 5 mg/kg/d and 25 mg/kg/d, respectively. Excessive amounts, on the other hand, might result in metabolic acidosis, seizures, asthma, and allergic responses, among other things (Chaojian *et al.,* 2019). Different countries have there on regulations, the addition of any preservatives to fruit juices is likewise prohibited by the Turkish Food Codex. The permissible daily intakes in Turkey for both preservatives were 0–5 mg benzoic acid intake/kg bodyweight and 0–25 mg sorbic acid intake/kg bodyweight. Similarly, the typical Portuguese population's ADIs for benzoic acid and sorbic acid are 0.25 mg intake/kg bodyweight and 0.17 mg intake/kg bodyweight, respectively, representing 4.9 percent and 0.68 percent of the ADI. Furthermore, the typical consumer's estimated benzoate and sorbate intakes in Brazil were found to be substantially below the ADIs, ranging from 0.3 to 0.9 mg/kg body weight and 0.2 mg/kg body weight to 0.3 mg/kg body weight, respectively (Cakir and Cagri-Mehmetoglu, 2014).

3.2. pH Measurement of Fruit juice-based Beverages

Table 2 exhibits the pH readings of fruit juice-based beverages tested, where it ranged from 3.09 to 4.26. Brand K tropical fruit juice drink base has the lowest pH value, and Brand Q lychee cordial has the highest pH value. On the other hand, Figure 2 illustrated the differences in pH values for five different categories of fruit juice-based beverages.

According to Table 2, Brand A cranberry and mixed fruit juice have the lowest pH value among all the fruit juices. The manufacturer of Brand A cranberry and mixed fruit juice has claimed that the particular product was made up of 55% grape juice, 30% apple juice, and lastly, 15% of cranberry juice. The presence of cranberry juice has lowered the overall pH of the beverage blend. It also indicated the differences in pH values between apple juices of two different brands. According to the beverages' respective labels, Brand B apple juice was not added with sugar while Brand E apple juice was added with cane sugar. In the absence of additional sugar, the pH value of Brand B apple juice was lower than that of Brand E apple juice. The pH reading for Brand C pineapple juice was 3.59, and it was similar to the pH value of the pineapple fruit fleshes studied by Bartolome *et al.* (1994), where the pH values for both Red Spanish and Smooth Cayenne cultivars of pineapple fruits were 3.49 and 3.54, respectively. The reason was that the manufacturer of Brand C pineapple juice produced the juices from fresh pineapple fruits, not from concentrate.

Brand K tropical fruit juice drink base has the lowest pH reading not only in the category of fruit juice drink but also among all the beverages experimented. Under the same category as Brand K products, Brand B orange juice drink without sugar has the highest pH value of 4.03. For fruit drink, there were two types of orange drinks with different brands. Even though both drinks were made from the same fruit juice type, the pH values were different. As presented in Table 2, Brand Q lychee cordial has the highest pH value of 4.26 among all the tested fruit cordials, while Brand R lemon cordial has the lowest pH value of 3.45.

The natural pH of fruits may differ depending on the fruit cultivar, cultivation practices, harvest season, maturity, and the handling of harvest and post-harvest (Bates *et al.,* 2001). Table 2 exhibits the pH readings and Figure 2 illustrated the differences in pH values for five different categories of fruit juice-based beverages. Fruit cordials were shown to have the highest pH value, followed by fruit juices, fruit nectars, fruit juice drinks, and lastly fruit drinks. Fruit cordials contained the highest level of sugar and can only be consumed upon dilution. Hence, their pH values would be higher as compared to ready-to-drink products. Wilbur and Ronald, (2001) stated that cranberry juice alone had the lowest pH value among all the other fruit juices. Due to its low pH and high tartness level, it was often blended with other types of fruit juice to produce beverage blends. Grape juice was found to be more acidic than orange juice and pineapple juice when the three liquids were compared. The pH dropped the most after drinking grape juice, followed by orange and pineapple juice, in that order (Mehta *et al.,* 2019).

Bates *et al.* (2001) also had pointed out that sour cherry juice was amenable to blends with less acidic juices or as nectar with added sugar due to its tartness level. Since sour cherry fruit was more acidic than other fruits used to make fruit nectar in this study, its pH reading was the lowest. A study by Grenby *et al.* (1989) has shown that the pH of orange drink and low-sugar orange drink were 2.7 and 3.5, correspondingly. Both orange drinks analysed in this study have higher pH values than the product samples. Lemon cordial was made from lemon juice. Since the natural pH of lemon fruit is lower than other fruits used to produce the tested fruit cordials, the pH of lemon cordial would be lower than other fruit cordials (Bates *et al.,* 2001; Wilbur and Ronald, 2001).

3.3.Titratable Acidity of Fruit juice-based Beverages

The titratable acidity for all the samples tested were shown in Table 2. The titratable acidity of fruit juice-based beverages ranged from 0.14 to 2.71% (w/v), with Brand O tropical fruit drink base having the highest percentage of predominant acid of 2.7% (w/v) while Brand Q lychee cordial having the lowest percentage of predominant acid of 0.1% (w/v). The percentages of predominant acid contained in five different categories of fruit juice-based beverages were shown in Figure 2. On average, the highest percentage of predominant acid was found in fruit juice drinks, followed by fruit drinks, fruit cordials, fruit juices, and lastly fruit nectars. For pineapple juice and cranberry and mixed fruit juice, the titratable acidity, calculated as anhydrous citric acid, shall not exceed 3.5% (w/v). Both of them did not violate the legal limit, Food Regulations 1985. Brand D pure orange juice did not violate the legal limit as it contained an average of 0.71 g of anhydrous citric acid in 100 mL. Values of Brand C pineapple juice, which contained only 0.64 g of acid per 100 mL.

In the group of fruit nectar product, sour cherry nectar tested contained the highest percentage value of acid with 0.79 % (w/v). The lowest acid content was found in guava nectar containing an average of 0.18 % (w/v) of anhydrous citric acid. As indicated in Table 2, Brand K tropical fruit juice drink base has the highest percentage value of predominant acid in the category of fruit juice drink. The percentage of acid presented in Brand B pink guava juice drink was the lowest as it contained only 0.28 % (w/v) of anhydrous citric acid. Brand O tropical fruit drink base containing 2.71 % (w/v) of anhydrous citric acid has the highest percentage of acid not only in the category of fruit drink product but also among all the beverages in the study. The lowest acid content was found in Brand M apple drink with 0.16 g of malic acid in 100 mL. On the other hand, Brand R lemon cordial contained the highest amount of anhydrous citric acid content with 1.94 % (w/v) due to the natural tartness level of lemon fruits (Bates *et al.*, 2001). Brand Q lychee cordial has the lowest percentage value of predominant acid, with only 0.14 % (w/v). As indicated in Table 2, the total acid contents for lychee cordials with two different brands were similar to each other.

Juices are liquids that many people take on a regular basis, with children being among the most avid users. Fruit and vegetable juices are made by extracting the natural liquid from the fruits or vegetables. The endogenous pH, titratable acidity, and ascorbic acid content of juices widely ingested by children are evaluated by Ogbeide *et al.* (2020). The Malaysian government has set the titratable acidity standard for fruit juices (Legal Research Board, 2010). No acidity specification was set for other categories of beverages studied. For apple juice, titratable acidity was calculated as malic acid. The value shall not be less than 0.3 g and not more than 0.8 g of acid in 100 mL measured at 20°C. Malaysian Food Regulations also stated that the titratable acidity of orange juice, calculated as anhydrous citric acid, shall contain not less than 0.65 g and not more than 1.5 g of acid in 100 mL. Bartolome *et al.* (1994) had researched the titratable acidity of fresh pineapple. The titratable acidity of Red Spanish pineapple and Smooth Cayenne pineapple were 1.2 and 0.9 grams of acid per 100 mL, respectively. The differences in processing, fruit cultivar, and fruit maturity level are the factors that cause these variations in pH (Bates *et al.*, 2001). Sour cherries are generally more acidic than peaches, guavas, and apricots (Bates *et al.*, 2001). For this category, the titratable acidity of concentrated fruit juice drink was higher than that of ready-to-drink products. According to Cairns *et al.* (2002), the titratable acidity of drinks was reduced as the drink became more dilute.

Table 1. Amount of benzoic acid and sorbic acid present in five different categories of fruit juice-based beverages

Category	Product name	Label Claim	Benzoic acid (ppm)*	Sorbic acid (ppm)*
Fruit juice	Cranberry and mixed fruit juice (Brand A)	No preservatives	6.1 ± 0.3^{bG}	ND^{bH}
	Apple juice (Brand B)	No preservatives	ND^{cG**}	ND^{bH}
	Pineapple juice (Brand C)	No preservatives	ND^{cG}	ND^{bH}
	Orange juice (Brand D)	No preservatives	ND^{cG}	ND^{bH}
	Apple juice (Brand E)	Permitted preservative	149.4 ± 0.6^{aF}	104.7 ± 0.7^{aEF}
Fruit nectar	Peach nectar (Brand F)	No preservatives	ND^{aG}	ND^{aH}
	Guava nectar (Brand G)	No preservatives	ND^{aG}	ND^{aH}
	Sour cherry nectar (Brand H)	No preservatives	ND^{aG}	ND^{aH}
	Apricot nectar (Brand I)	No preservatives	ND^{aG}	ND^{aH}
	Multivitamin 12 fruit nectar (Brand J)	No preservatives	ND^{aG}	ND^{aH}
Fruit juice drink	Orange juice drink without sugar (Brand B)	Permitted preservative	ND^{dG}	238.4 ± 2.3^{aB}
	Pink guava juice drink (Brand B)	Permitted preservative	ND^{dG}	182.3 ± 1.0^{bC}

	Tropical fruit juice drink base (Brand K)	Permitted preservatives (contains sulfur dioxide)	690.0 ± 2.8^{aA}	ND^{cH}
	Blackcurrant fruit juice drink base (Brand K)	Permitted preservatives (contains sulfur dioxide)	484.5 ± 3.8^{bB}	ND^{cH}
	Pomegranate and apple juice drink (Brand L)	Sodium benzoate (E211)	232.2 ± 1.4^{cE}	ND^{cH}
Fruit drink	Apple drink (Brand M)	No preservatives	ND^{bG**}	ND^{dH}
	Orange drink (Brand D)	Permitted preservative	ND^{bG}	256.9 ± 1.1^{aA}
	Orange drink (Brand N)	Permitted preservative	ND^{bG}	79.8 ± 1.0^{cG}
	Tropical fruit drink base (Brand O)	Permitted preservatives	312.4 ± 3.4^{aD}	148.7 ± 3.2^{bD}
	Tropical mixed fruit drink (Brand P)	No preservatives	ND^{bG}	ND^{dH}
Fruit cordial	Lychee cordial (Brand Q)	Permitted preservative	ND^{dG}	ND^{cH}
	Lemon cordial (Brand R)	Sodium metabisulphite (E223)	ND^{dG}	ND^{cH}
	Mixed fruit cordial (Brand R)	Sodium benzoate (E211) and sodium metabisulphite (E223)	494.3 ± 4.3^{aB}	ND^{cH}
	Lychee cordial (Brand S)	Permitted preservatives	382.0 ± 4.2^{cC}	106.9 ± 2.7^{aE}
	Mango cordial (Brand T)	Permitted preservative	469.6 ± 4.2^{bB}	95.9 ± 2.9^{bF}

* Average ± S.E.M. of three determinations.
** ND, no detection
$^{a-e}$ Means with different letters within the same category were significantly different at $p \leq 0.05$.
$^{A-H}$ Means with different letters between categories were significantly different at $p \leq 0.05$.

Table 2. Physicochemical properties of fruit juice-based beverages

Category	**Product name**	**pH***	**Predominant acid**	**Percentage of acid (%)** *	**Brix value (°Brix)** *	**Brix-to-acid ratio***
Fruit juice	Cranberry and mixed fruit juice (Brand A)	3.31± 0.01^{eK}	Citric acid	0.41 ± 0.00^{cH}	14.5 ± 0.0^{aI}	35.5 ± 0.3^{aG}
	Apple juice (Brand B)	3.63 ± 0.01^{cF}	Malic acid	0.39 ± 0.01^{dH}	11.8 ± 0.1^{dK}	32.1 ± 0.3^{cG}
	Pineapple juice	3.59 ±	Citric acid	0.64 ±	14.2 ±	22.3 ±

	(Brand C)	0.01^{dG}		0.00^{bF}	0.0^{bJ}	0.2^{dH}
	Orange juice (Brand D)	4.01 ± 0.02^{aB}	Citric acid	0.71 ± 0.00^{aE}	12.0 ± 0.0^{cK}	16.9 ± 0.1^{eI}
	Apple juice (Brand E)	3.72 ± 0.01^{bD}	Malic acid	0.33 ± 0.00^{eHI}	11.0 ± 0.0^{eL}	34.5 ± 0.3^{bG}
Fruit nectar	Peach nectar (Brand F)	3.73 ± 0.01^{BD}	Citric acid	0.31 ± 0.00^{cI}	13.8 ± 0.0^{cJ}	44.4 ± 0.3^{bF}
	Guava nectar (Brand G)	3.96 ± 0.02^{aC}	Citric acid	0.18 ± 0.00^{dJ}	14.7 ± 0.1^{aI}	80.5 ± 2.3^{aCD}
	Sour cherry nectar (Brand H)	3.20 ± 0.03^{dM}	Malic acid	0.79 ± 0.00^{aE}	13.0 ± 0.0^{dJ}	17.3 ± 0.1^{dI}
	Apricot nectar (Brand I)	3.71 ± 0.01^{bD}	Malic acid	0.32 ± 0.00^{cI}	14.1 ± 0.0^{bJ}	45.5 ± 0.3^{bF}
	Multivitamin 12 fruit nectar (Brand J)	3.55 ± 0.02^{cH}	Citric acid	0.49 ± 0.00^{bG}	11.8 ± 0.0^{eK}	24.0 ± 0.2^{cH}
Fruit juice drink	Orange juice drink without sugar (Brand B)	4.03 ± 0.01^{aB}	Citric acid	0.53 ± 0.00^{cG}	10.2 ± 0.0^{eL}	19.2 ± 0.1^{eHI}
	Pink guava juice drink (Brand B)	3.72 ± 0.02^{bD}	Citric acid	0.28 ± 0.00^{eI}	11.4 ± 0.0^{dK}	40.7 ± 0.1^{aFG}
	Tropical fruit juice drink base (Brand K)	3.09 ± 0.01^{eN}	Citric acid	2.40 ± 0.03^{aB}	60.9 ± 0.1^{aA}	25.4 ± 0.3^{dGH}
	Blackcurrant fruit juice drink base (Brand K)	3.37 ± 0.01^{dJ}	Citric acid	1.31 ± 0.02^{bD}	52.0 ± 0.1^{bC}	39.7 ± 0.7^{bFG}
	Pomegranate and apple juice drink (Brand L)	3.63 ± 0.01^{cF}	Malic acid	0.39 ± 0.01^{dH}	13.2 ± 0.0^{cJ}	35.6 ± 0.9^{cG}
Fruit drink	Apple drink (Brand M)	3.36 ± 0.01^{dJ}	Malic acid	0.16 ± 0.00^{cJ}	12.2 ± 0.0^{bK}	81.3 ± 0.0^{aC}
	Orange drink (Brand D)	3.61 ± 0.01^{aFG}	Citric acid	0.28 ± 0.01^{bcI}	12.6 ± 0.1^{bK}	44.8 ± 0.6^{cF}
	Orange drink (Brand N)	3.55 ± 0.01^{cH}	Citric acid	0.32 ± 0.00^{bHI}	11.8 ± 0.1^{bK}	37.5 ± 0.5^{dG}
	Tropical fruit drink base (Brand O)	3.24 ± 0.02^{eL}	Citric acid	2.71 ± 0.10^{aA}	60.1 ± 0.1^{aB}	22.2 ± 0.8^{eH}
	Tropical mixed fruit drink (Brand P)	3.57 ± 0.01^{bG}	Citric acid	0.19 ± 0.00^{cJ}	11.6 ± 0.1^{bK}	59.5 ± 1.0^{bE}
Fruit cordial	Lychee cordial (Brand Q)	4.26 ± 0.01^{aA}	Malic acid	0.14 ± 0.01^{cJ}	37.4 ± 0.0^{dG}	275.7 ± 13.7^{aA}
	Lemon cordial (Brand R)	3.45 ± 0.01^{dI}	Citric acid	1.94 ± 0.04^{aC}	41.0 ± 0.1^{cF}	21.2 ± 0.5^{dHI}

	Mixed fruit cordial (Brand R)	3.67 ± 0.01^{bE}	Citric acid	0.60 ± 0.02^{bE}	41.6 ± 0.1^{bE}	69.2 ± 2.2^{cD}
	Lychee cordial (Brand S)	4.23 ± 0.01^{aA}	Malic acid	0.17 ± 0.01^{cJ}	32.4 ± 0.1^{eH}	200.6 ± 13.2^{bB}
	Mango cordial (Brand T)	3.60 ± 0.01^{cGF}	Citric acid	0.63 ± 0.02^{bF}	50.6 ± 0.0^{aB}	80.5 ± 2.7^{cC}

$^{a-e}$ Means with different letters within the same category were significantly different at $p \leq 0.05$.
$^{A-N}$ Means with different letters between categories were significantly different at $p \leq 0.05$.

Table 3. Label instructions of dilution for fruit cordials found in respective sample labels

Sample name	Dilution instruction on the label
Lychee cordial (Brand Q)	1 part of cordial to 5 parts of water
Lemon cordial (Brand R)	1 part of cordial to 4 parts of water
Mixed fruit cordial (Brand R)	1 part of cordial to 4 parts of water
Lychee cordial (Brand S)	1 part of cordial to 4 parts of water
Mango cordial (Brand T)	1 part of cordial to 7 parts of water

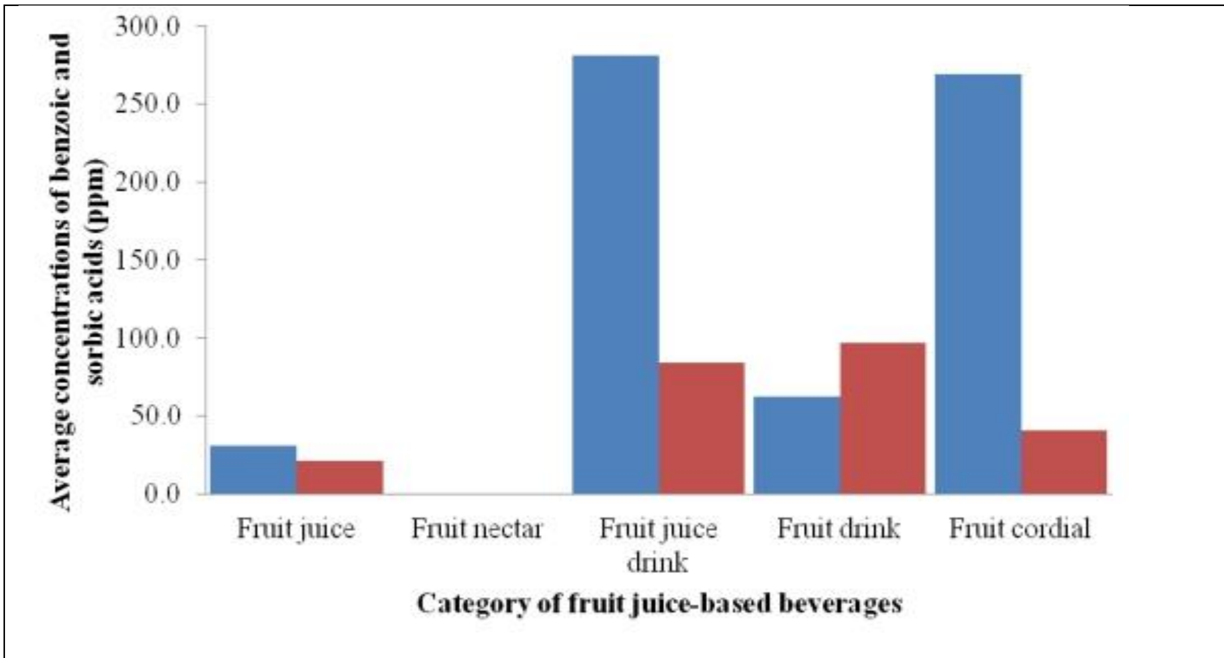

Figure 1. The concentrations of benzoic (blue) and sorbic acids (red) (ppm) found in five different categories of fruit juice-based beverage

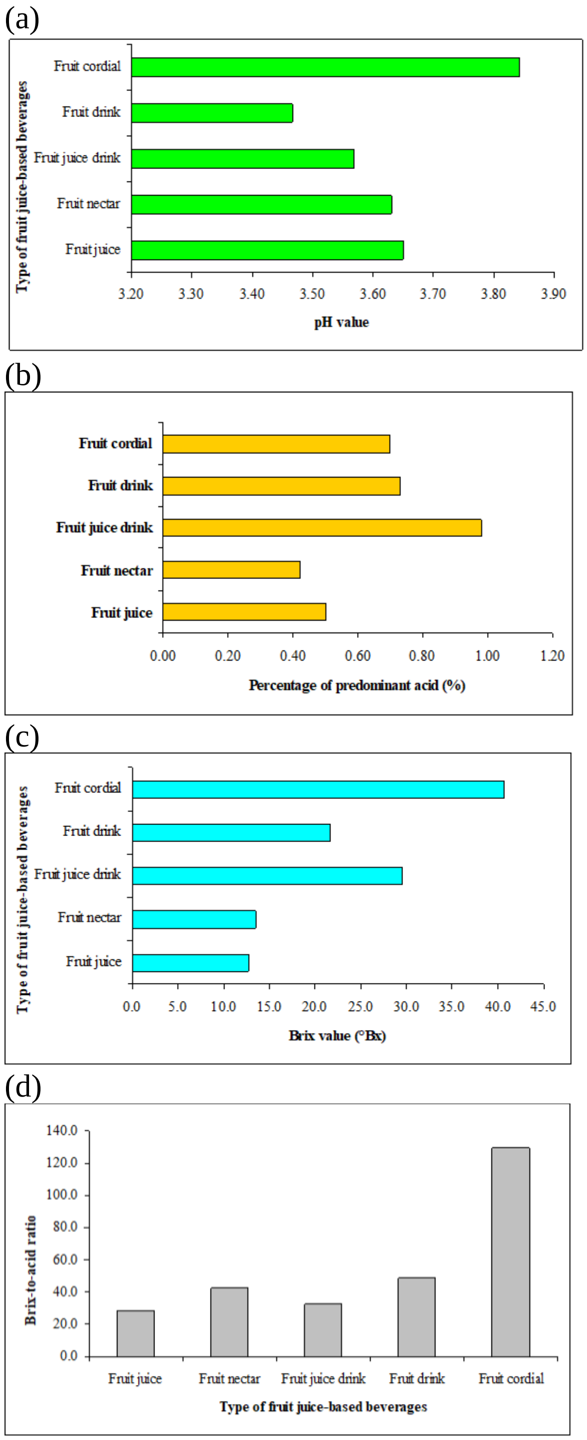

Figure 2. pH values (a), percentages of predominant acid (b), Brix (c) and Brix-to-acid ratios (d) of five different kinds of fruit juice-based beverages

3.4.The Brix value of Fruit juice-based Beverages

Table 2 presented the Brix values for five different categories of fruit juice-based beverages measured by using handheld refractometers. On average, Brand B orange juice drink without sugar has the lowest Brix value, which was 10.2 °Brix. Brand K tropical fruit juice drink base product was in the same group as Brand B orange juice drink but was marketed in the form of concentrate. It has the highest Brix value of 60.9 °Brix among all the samples tested. The Brix values for all the fruit juices tested ranged from 11.0 to 14.5 °Brix with the highest value found in Brand A cranberry and mixed fruit juice, and the lowest value found in Brand E apple juice. Brand E apple juice did not comply with the requirement as its value was below the minimum level imposed by Food Regulations 1985. The soluble solids content of orange juice was established not to be less than 10.5 g in 100 mL. Since Brand D orange juice, Brand A cranberry and mixed fruit juice and Brand C pineapple juice also did not violate the legal limits of Food Regulations 1985 as their Brix levels have exceeded the minimum percentage required. The Brix value must be more than 11.2°Brix for fruit juices made from concentrates. Brand B apple juice was following the specification, while the Brix level of Brand E apple juice was lower than the requirement. In the category of fruit nectar, the Brix levels of samples ranged from 11.8 to 14.7 °Brix. Mostly products label claim has complied with the labelling regulation of Food Regulations 1985.

As shown in Table 2, the highest Brix value of 50.6 °Brix was detected in Brand T mango cordial, and the lowest Brix value of 32.4 °Brix was detected in Brand S lychee cordial. Table 3 showed the label instructions of dilution for five different types of fruit cordials. According to the label instructions, a higher amount of water is required to dilute Brand T mango cordials to prepare their ready-to-drink soft drinks. This means that the dilution factor was the highest in Brand T mango cordials. Brand Q and Brand S fruit cordials were made from the same type of fruit juices. However, their Brix values were different from each other. The Brix level of Brand Q lychee cordial was higher than that of Brand S lychee cordial because a higher amount of water was needed to dilute the cordial.

Average Brix readings for five different kinds of fruit juice-based products were given in Table 2. Among all the 75 samples tested, fruit cordials have the highest average Brix value, followed by fruit juice drinks, fruit drinks, fruit nectars, and lastly fruit juices. Fruit cordials are concentrated products. When water is removed from beverages, the juice solid will gradually increase up to 10-fold (Bates *et al.,* 2001), and therefore increases the total soluble solid contents. According to Food Regulations 1985, apple juice shall contain not less than 11.5 g of soluble solids in 100 mL measured at 20 °C. CODEX general standard for soluble solids is classified into two: Brix level for reconstituted juice from concentrate and Brix level for single strength juice not from concentrate. The minimum Brix level requirement set by CODEX commodity committees was applicable only for single strength juice. Therefore, Brand A product containing different types of fruit juices was not compared with CODEX general standard. Under the current Food Regulations 1985, the total soluble solids content of fruit nectar shall not be less than 12 %. All of the fruit nectars tested were conformed to their specification limit. The minimum Brix level established by CODEX commodity committees for orange juice and pineapple juice is 10.0 °Brix and 11.2 °Brix, correspondingly. Both Brand C pineapple juice and Brand D orange juice have met the quality requirement.

The Brix levels for all the tested fruit juice drinks ranged from 10.2 to 60.9. Tropical fruit juice drink base has the highest Brix value, and orange juice drink without sugar has the lowest Brix value. Taylor (2007) has pointed out that Brix value was related directly to both the sugars and fruit acids. Since the percentage of fruit juice is not high in fruit juice drinks and the effect of fruit acids is not significant, the amount of added sugar becomes the main contributor to the Brix level. Fruit juice drinks are soft drinks. In Malaysia, the minimum Brix level required for soft drinks is not specified. Hence, no comparison was made between all the soft drinks and Food Regulations 1985. While for fruit drinks, the highest Brix value was found in tropical fruit drink base product as it was the only fruit drink which marketed in concentrated form. Other fruit drinks were sold in prepared forms. All the fruit cordials were manufactured in different concentration levels by different beverage companies. The differences in concentration of fruit cordials were compared by reading their respective label instructions for dilution.

3.5. The Brix-to-Acid ratio of fruit Juice-Based Beverages

The Brix-to-acid ratios of fruit juice-based products were presented in Table 2. According to the table, Brand D orange juice has the lowest ratio value of 16.9. Hence, its taste would be sourer as compared to others. Brand Q lychee cordial has the highest Brix-to-acid ratio of 275.7. Its sweetness taste was the highest among all the products tested. Figure 2 also shows the differences in the Brix-to-acid ratios between five different kinds of fruit juice-based beverages.

The Brix-to-acid ratios of fruit juices ranged from 16.9 to 35.5, with the highest value found in Brand A cranberry and mixed fruit juice and the lowest value found in Brand D orange juice. Food Regulations 1985 has specified the standard for the Brix-to-acid ratio of orange juice. The Brix-to-acid ratios for other types of fruit juice were not included in the standard. Cranberry juice provides tartness mouth feel to consumers who drink it. The strong sour sensation of pure cranberry juice may reduce consumer preferences towards the product. Therefore, it is more commonly blended with other types of fruit juice. For instance, the cranberry juice in Brand A product was blended with apple and grape juices.

Among all the fruit nectars, guava nectar has the highest sugar-to-acid ratio value. Its value was much higher than its counterparts of the same group. Sour cherry nectar has the lowest Brix-to-acid ratio. Fructose syrup was one of the ingredients added in Brand H sour cherry nectar. It played an important role in increasing the Brix-to-acid ratio of the product to an acceptable level. On the other hand, in the group of fruit juice drink, Brand B pink guava juice drink has the highest Brix-to-acid ratio, and Brand B orange juice drink has the lowest Brix-to-acid ratio. Sugar was not added to the Brand B orange juice drink. Thus, its Brix value was the lowest in this group.

The sugar-to-acid ratios of fruit drinks ranged from 22.2 to 81.3, with the highest value found in Brand M apple drink and lowest value found in Brand O tropical fruit drink base. Table 2 has also shown that the Brix-to-acid ratios differed for orange drinks with different brands. Brand Q lychee cordial has the highest Brix-to-acid ratio of 275.7. Both Brand Q and Brand S lychee cordials have very high sugar-to-acid ratios as compared to other fruit cordial samples. The lowest sugar-to-acid ratio was found in Brand R lemon cordial with only 21.2. Lemon juice was more acidic as compared to other fruit juices contained in fruit cordials tested. As a result, its high percentage of acid has reduced the sugar-to-acid ratio.

Generally, the acidity of fruit juices would decrease with increasing maturity of fruits, or with increasing levels of sugars in resulting juice (Taylor, 2007). In the beverage industry, the Brix-to-acid ratio could be used to establish standard sensory, maintain the qualities of products, and also to minimize the effect of seasonal variation. The higher the Brix value as compared to the acid content of the juice, the higher the ratio value and the sweetness taste would increase as well. Fruit cordials have the highest average Brix-to-acid ratio value, followed by fruit drinks, fruit nectars, fruit juice drinks, and lastly fruit juices. This is because fruit cordials are concentrated products. Since their degree Brix values will be much higher than other ready-to-drink fruit juice-based products, the ratio of Brix to acid will be much higher. By using the blending method, the sugar-to-acid ratio can be adjusted to a value that meets consumer demand (Bates *et al.,* 2001). According to Bates *et al.* (2001), cherry cultivars range from extremely sour to low sweet acid with sugar-to-acid ratios from 7 to 35. The Brix value would then lower the average sugar-to-acid ratio.

According to the United States Code of Federal Regulations, juices extracted straight from a fruit or vegetable are considered 100% juice and must be stated as such. When reconstituted from juice concentrate, however, the US FDA defines 100% juice as Brix concentrations that are typical of those extracted from the fruit. Physical qualities including Brix concentration, acidity, Brix:acid ratio, colour, and flavour all have an impact on overall 100% juice quality (Roger *et al.*, 2015).

4. Conclusions

It was concluded that among all the 75-fruit juice-based beverage samples, 15 samples were found to contain benzoic acid with Brand K tropical fruit juice having the highest content, while 12 samples were found to contain sorbic acid. Brand D orange juice that contains highest content of sorbic acid. A preference for incorporating benzoates (cheaper price) over sorbates into fruit juice-based beverages were noted. A combination of benzoic and sorbic acids was detected in 12 samples for synergistic effect of bacterial inhibition, and the remaining 36 samples did not contain any benzoic acid or sorbic acid. All the fruit juice-based beverages studied did not violate the legal limit for benzoic acid or sorbic acid imposed by Food Regulations 1985 and no violation of the labelling requirement was observed. In general, physicochemical properties of fruit juice-based beverages such as pH, titratable acidity, total soluble solids content, and sugar-to-acid ratio were affected by factors such as fruit, specifications and company. Therefore, physicochemical properties may differ for products made of the same fruit juice type. Also, the natural pH of lemon and cranberry fruits was lower than other fruits. Thus, their physicochemical properties' values were most likely to be lower than other fruit juice-based beverages.

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