Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

# Total fructose, total glucose and benzoic acid contents in commercially available fruit nectars in Anuradhapura town area, Sri Lanka

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ARTICLE INFO

*Article history:*
Received 23 Sep. 2021
Received in revised form
17 Dec. 2021
Accepted 24 Dec. 2021

***Keywords:***
***Benzoic acid;***
***Free fructose;***
***Free glucose;***
***Fruit nectar***

ABSTRACT

High intake of total fructose, total glucose and benzoic acid can cause many adverse health effects. Most commercially available fruit juice and nectar brands in Sri Lanka are not claimed on the label about the levels of fructose, glucose and benzoic acid in the products. Available brands of fruit juices [Mixed Fruit Juice (MFJ) and Mango Juice (MJ)] and fruit nectars [Mixed Fruit Nectar (MFN) and Mango Nectar (MN)] were selected through a pre-validated questionnaire. Seven brands of MN and MFN were identified as mostly consumed and there was a significant difference *($p<0.05$)* in the total fructose and total glucose levels among the brands. Total fructose of MN and MFN was in the range of 28.54 ± 3.03 g/l to 60.28 ± 5.04 g/l and 30.46 ± 8.56 g/l to 62.47 ± 9.27 g/l respectively. Total glucose content of MN was in the range of 17.49 ± 5.39 g/l to 41.87 ± 2.11 g/l and for MFN from 29.29 ± 4.34 g/l to 85.71 ± 5.64 g/l. Total fructose in MJ and MFJ was in the range of 32.28 ± 0.86 g/l to 37.66 ± 0.55 g/l and 33.14 ± 0.87 g/l to 36.81 ± 1.81 g/l respectively. Three, out of the four brands of MN and MFN contained benzoic acid. One brand of MN and one brand of MFN had exceeded the permitted level (120 mg/l) of benzoic acid. Benzoic acid was present in two, out of three brands of MJ and MFJ. It can be concluded that there is a variation in total fructose, total glucose and benzoic acid contents in fruit nectars and juices available for sale in Anuradhapura town area. However, some brands have violated the Sri Lanka food regulations.

**Citation:** Hettiarachchi RL, Senadheera S, Gihan Erandika Wijelath WA, Gayan Chathuranga Thilakarathana HM. **Total fructose, total glucose and benzoic acid contents in commercially available fruit nectars in Anuradhapura town area, Sri Lanka.** J food safe & hyg 2021; 7(4): 226-236

## 1. Introduction

Fruits help to maintain good health by preventing some diseases in humans (8). They are rich in fiber, sugars, vitamins, polyphenols, antioxidants, carotenoids and flavonoids (21). Therefore, fruit-based drinks have garnered higher attention as a healthy drink when compared to other soft drinks. Fruit nectars (ready to serve without dilution) are mostly consumed as fruit drinks (23).

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They are ready to consume, sweet in taste, easily digestible and has refreshing and thirst quenching ability (23,27). Thus, instead of fresh fruits, consumption of fruit nectar is becoming very popular among consumers as these beverages can be carried easily everywhere and as they have long shelf life (4). Preparation of fruit nectar is a value addition process and a preservative mode. Further it reduces postharvest losses.

Fruit based beverage consumption elicit a significant upward trending owing to the health advantages and ease of consumption. Ready-to-drink fruit drink market in Sri Lanka values almost 12 million USD. Annual growth rate of the fruit drink market shows an exponential growth of 12% (17).

The sugars in fruit based beverages; free fructose and glucose have massive impact on human health. Fructose intake somewhere in the range of 0 and 100 g for each day does not show a significant hazard on human health. However, excess consumption of fructose and glucose has proven associations with the occurrence of diabetes mellitus, obesity and non-alcoholic fatty liver disease (5).

Food preservatives cause health issues in humans. Some countries have banned the use of certain preservatives in the fruit drink industry (24). Benzoic acid is one of the most commonly used chemical preservatives in the beverage industry as an antifungal agent (13). Benzoic acid is added to fruit drinks in the form of sodium benzoate, potassium benzoate or calcium benzoate (26).

Even though Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommend benzoic acid as a food additive; many studies claim that benzoate may cause allergic reactions in sensitive people, especially when used with food colorants (3). In the presence of ascorbic acid and metals, benzoate is converted to benzene. This reaction is accelerated by light and heat. International Agency of Research on Cancer (IARC) classified benzene as a group 1 human carcinogen. Further, utilization of glycine for the detoxification of benzoate results in a reduction of glycine level in human body. Long term consumption of benzoic acid and its salt affect body functions and metabolic processes where glycine is involved (WHO, 1996).

This study aimed to assess the amount of free fructose, free glucose and benzoic acid content in mostly consumed brands of fruit nectars commercially available in Anuradhapura town area, Sri Lanka.

## 2. Materials and Methods

### 2.1. Selection of Highly Consumed Brands

A pre-validated self-administered 50 questionnaire were given to vendors of main supermarkets and retail outlets in Anuradhapura town area. The questionnaire included basic information such as type of the shop, location, selling items and other information relevant to the study (brands, fruit nectar types and flavors, amounts of sales per week). Mostly consumed seven fruit nectar brands and two flavors were identified based on the market survey data.

The fruit nectar was purchased from 50 places in Anuradhapura town area following random sampling method. They were transported to the laboratory as soon as possible and kept under refrigerated condition at 4$^{o}$C.

2.2. Analysis of Free Fructose, Free Glucose and Benzoic acid

All the laboratory experiments and analysis were carried out in the Biochemistry Research Laboratory, Department of Biochemistry, Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka.

2.3. Determination of Free Fructose

Free fructose content was determined by using Resorcinol regent ($C_6H_6O_2$) (27).

2.4. Preparation of Samples

Fruit nectar sample was diluted by 1:50 ratio and 2 ml of the diluted sample was added to 1 ml of newly prepared Resorcinol regent ($C_6H_6O_2$).

2.5. Preparation of the Calibration Curve

Standard fructose 100 mg was dissolved in 100 ml distilled water and served as the stock solution for the standards. Stock solution 10 ml was diluted with distilled water up to 100 ml to serve as the working standard solution. From the working standard 0, 2, 4, 6, 8 and 10 ml were pipetted and 1 ml of Resorcinol regent was added to each test tube. All the test tubes were heated in a water bath at 80$^{o}$C for 20 min. Tubes were allowed to cool up to room temperature and placed in the UV-VIS spectrophotometer. Absorbance values were noted at 520 nm within 30 min and a graph; absorbance vs. concentration was plotted. Fructose concentration of the fruit nectar was calculated using the equation of the standard curve.

2.6. Determination of Free Glucose

Glucose content was determined using a glucose oxidase colorimetric assay kit (02160, BIOLABO Maizy, France). Phosphate buffer (150 mg/l), Glucose oxidase (GOD)≥20000 UI/l, Peroxidase (POD)≥1000 UI/l and 4-Amino- antipyrine (PAP) 0.8 mmol/l were the constituents of the assay reagent. Concentration of the standard glucose regent was 100 mg/dl (5.55 mmol$^{-1}$). The chromogen was Chloro-4- phenol (2 mmol/l).

2.7. Determination of Benzoic acid

Nectar samples belonged to 4 selected brands (n=12 from each brand) were purchased from selected points in Anuradhapura town area. Benzoic acid content was determined according to the method by using UV-Visible spectrophotometer (14) with some modifications. Samples stored exposed to sunlight and sample stored without exposing to sunlight were collected from each brand and this storage condition was considered during the analysis.

2.8. Preparation of Samples

From the 1:10 diluted sample, 4 ml was added to a 100 ml volumetric flask and 10 ml of 0.10 M HCL was added. Distilled water was added up to 100 ml mark. Stock solution was prepared using 100 mg of benzoic acid dissolved in 100 ml of distilled water. Standard curve of benzoic acid was used to determine the Benzoic acid concentration of the fruit nectar samples. Absorbance was measured at 273 nm using UV-Visible spectrophotometer.

2.9. Reduction of Benzoic Acid with the Effect of Sunlight

Samples were obtained from selected retail outlets where fruit nectars (Mango and mixed fruit) stored exposure to sunlight and supermarkets which stored in cool dry place. Effect of the sunlight towards the benzoic acid content in fruit beverages were estimated by measuring the benzoic acid content in nectars of same brand with same production dates which stored in cool dry places (supermarkets) and stored exposed to sunlight (selected retail outlets) in Anuradapura town area.

2.10. Data Analysis

Presence of any significant difference in glucose and fructose contents among samples, at 95% confident interval was determined by Analysis of Variance (ANOVA), GLM (General Lenear Model), Turkey's Standardized Range Test (TSRT), in Minitab software (version 17.1, Minitab Ltd., 2017).

Presence of a significant difference in benzoic acid at 95% confident interval was determined by t-test using Statistical Analysis Software (SAS) (version 9.4, SAS Institute Inc, USA, 2010)

## 3.Results

3.1.Consumer Preference

According to the market survey carried out in the Anuradhapura town area, percentage of the consumer preference for different brands of fruit nectars and the consumer's flavor preferences for each brand are shown in Fig. 1 and Fig. 2 respectively.

According to Fig. 1, out of A to M brands, A, B, D, E, H, F, M, were the mostly consumed brands of fruit nectars. Brand B had the highest consumer preference and the brand I and K had the lowest.

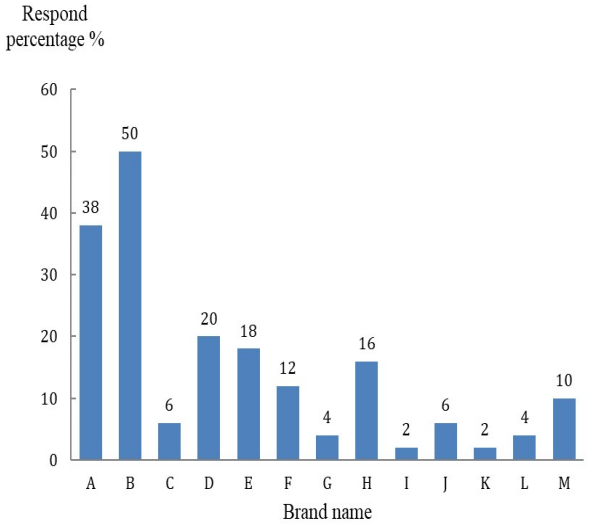


**Figure 1.** Consumer preference for different brands of fruit nectars in Anuradhapura town area (Different brands are coded as A, B, C, D, E, F, G, H, I, J, K, L and M)

Mango, mixed fruit, wood apple, orange and apple were noted as the flavors available under the mostly consumed brands of fruit nectars (Fig. 2). Mango and mixed fruit flavors were found as the mostly consumed flavors of fruit nectars.

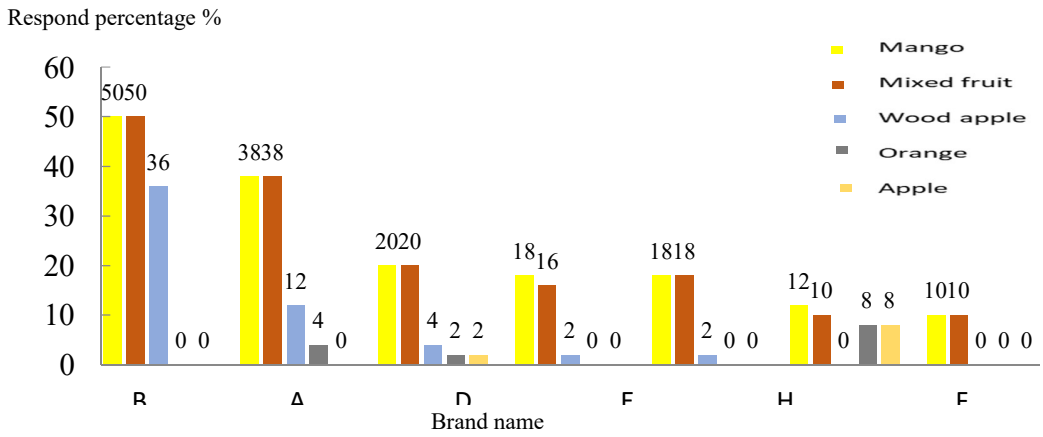


**Figure 2.** Flavor preferences of mostly available brands (A, B, D, E, H and F) of fruit nectars in Anuradhapura town area.

**Table 1.** Free fructose and glucose content (g/200 ml) in selected brands of mango nectar and mixed fruit nectar in Anuradhapura town area

| Brand Name | Free glucose content g per 200 ml | | Free fructose content g per 200 ml | |
|---|---|---|---|---|
| | Mango | Mixed fruit | Mango | Mixed fruit |
| A | $6.85^{b}$±0.14 | $5.86^{e}$± 0.87 | $12.06^{a}$ ± 0.24 | $12.36^{a}$ ± 0.30 |
| B | $8.37^{a}$±0.09 | $8.39^{b}$±0.58 | $5.71^{d}$± 0.14 | $6.09^{c}$± 0.40 |
| D | $3.49^{e}$±1.07 | $17.14^{a}$±1.12 | $8.73^{c}$±0.57 | $7.78^{b}$± 0.72 |
| E | $5.93^{d}$±0.61 | $8.80^{b}$±1.01 | $12.71^{a}$± 0.32 | $12.49^{a}$± 0.43 |
| F | $6.06^{c}$±0.10 | $7.00^{d}$±0.43 | $11.38^{b}$±0.30 | $11.81^{a}$±0.53 |
| H | $8.27^{a}$±0.55 | $7.67^{c}$±0.42 | $9.24^{c}$± 0.10 | $7.49^{c}$± 0.42 |
| M | $5.29^{d}$±0.26 | $8.72^{b}$±0.44 | $8.57^{c}$ ± 0.27 | $9.69^{b}$± 0.17 |

Values in the table shown as mean ± standard deviation of three replicate. The value with different superscripted letters in the same column are significantly different to each other ($p<0.05$).

3.2. Free Fructose and Free Glucose Content

Free glucose and fructose contents in selected brands of mango and mixed fruit flavored nectars are given in table 1.

According to the table 1, Br and E mango nectar had significantly high ($p<0.05$) amount of fructose content per serving while brand B had the lowest. Brand E and A mixed fruit nectars had no significant difference ($p>0.05$) and these two brands (E and A) had the highest amount of free fructose content per serving.

There was a significant difference ($p<0.05$) in free glucose content among the brands and between mango and mixed fruit nectars. Mango nectar of brand H had significantly ($p<0.05$) high free glucose content while brand D had the lowest.

3.3. Benzoic Acid Content

Benzoic acid content in fruit nectars are shown in table 2. According to table 2, brand B, D and H (both mango and mixed fruit nectars) contained benzoic acid. However, the presence of benzoic acid was not indicated on the labels of these three brands. There was a significant difference ($p<0.05$) in benzoic acid content in brand D, H of mango nectar and brand B, D and H of mixed fruit nectar as per serving. According to the Sri Lankan food preservative regulations maximum permitted level of benzoic acid for 200 mL is 0.032 g (160 ppm). Mixed fruit nectar of brand B, D and H had benzoic acid levels which exceed the permitted level. Mango nectar of these three brands contained the permitted level of benzoic acid.

**Table 2.** Benzoic acid content (g per 200 ml) in fruit nectars

| Brand name | Benzoic acid content g per 200 ml | |
|---|---|---|
| | **Mango nectar** | **Mixed fruit nectar** |
| B | $0.017^{b}$±0.007 | $0.052^{a}$±0.003 |
| D | $0.018^{b}$±0.001 | $0.182^{b}$±0.005 |
| H | $0.030^{a}$±0.055 | $0.108^{c}$±0.011 |
| M | ND | ND |

Values in the table shown as mean ± standard deviation of three replicate. The value with different superscripted letters in the same column is significantly different to each other ($p<0.05$); ND – not detected.

3.4. Reduction of benzoic acid with the effect of sunlight

According to Figure. 3, brand D (180.87 ppm) and B (53.88 ppm) of mango nectar which had been exposed to sunlight showed a significant reduction in benzoic acid level ($p<0.05$) compared to the same brands where nectars were kept without exposing to sunlight Brand H of mango nectar which had been stored without exposing to sunlight, had 118.56 ppm of benzoic acid level and a reduction up to 17.81 ppm was observed with the effect of sunlight.

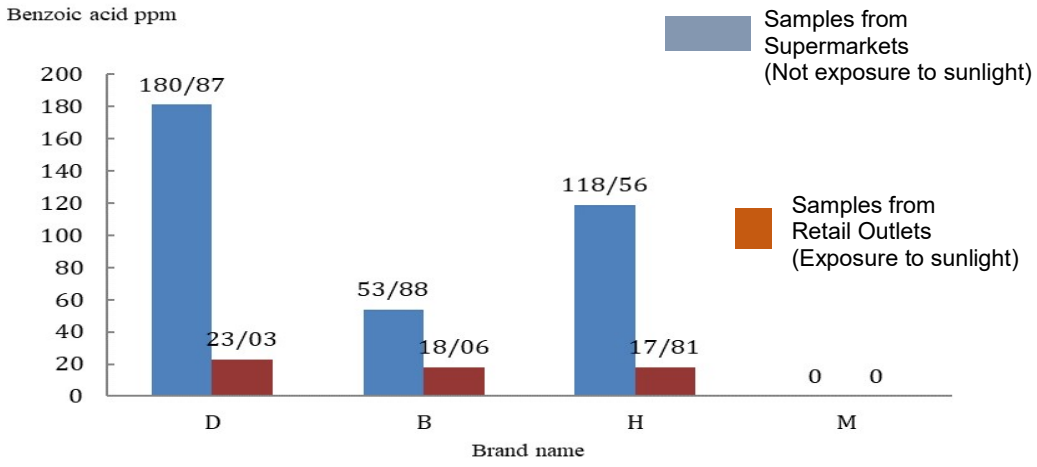


**Figure 3.** Reduction of benzoic acid in mango nectar with the effect of sunlight

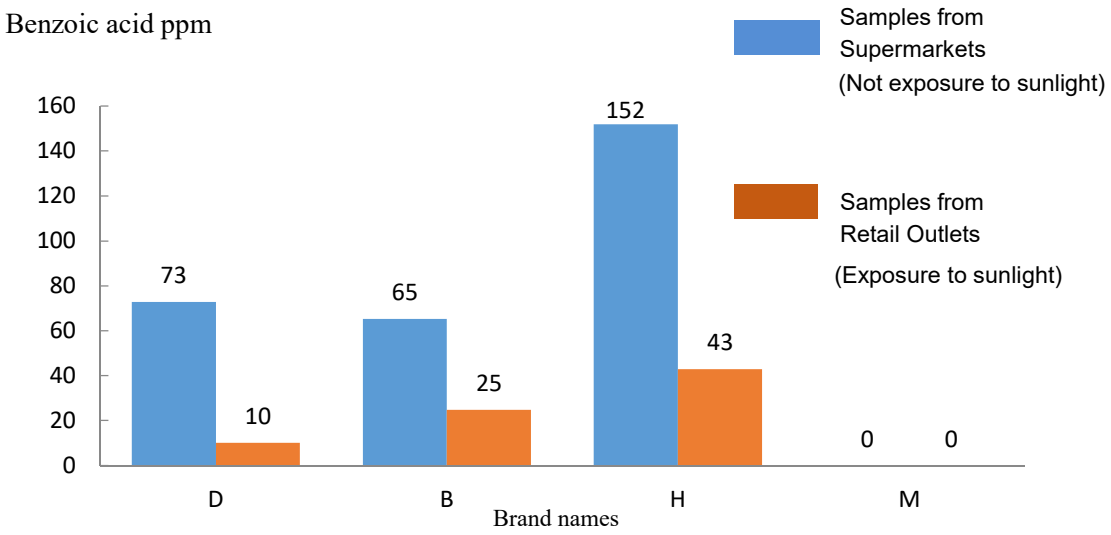


**Figure 4.** Reduction of benzoic acid in mixed fruit nectar with the effect of sunlight

**4.Discussion**

Significant differences in glucose and fructose levels in fruit nectars and juices tested in the present study indicate that most of these drinks contain added glucose or fructose or both.

Different levels of free fructose content in different brands could be attributed to the type of fruit pulp, added sugars and the manufacturing procedures. During large scale manufacturing procedures, some amount of fructose is added to the fruit pulp in the form of high fructose corn syrup (HFCS). HFCS contains 42% of fructose and 45% of glucose. Very high fructose levels in certain nectars could be due to the addition of HFCS. Moreover, glucose syrup is also added during manufacturing procedure, to increase the sweetness.

Fruit juices and nectars, containing more added glucose and fructose than naturally present could cause health complications. High blood glucose level is directly linked with diabetes and cardiovascular diseases (18) and fructose is atherogenic leading to many non-communicable diseases (10).

Fructose bypasses the main regulatory step of glycolysis (the conversion of glucose-6-phosphate to fructose 1, 6-bisphosphate). During this pathway a portion of triose-phosphate produced from fructose (fructose1, 6-bisphospte) can subsequently be converted into pyruvate and oxidized into $CO_2$ and water. Another portion is converted into lactate to be released into the circulation. The major portion of the triose-phosphate produced from fructose is converted into glucose through gluconeogenesis. At the end, part of the carbons from fructose can be converted to fatty acids. Simultaneously, fructose inhibits hepatic lipid oxidation favoring fatty acid re-esterification and Very Low Density Lipoprotein (VLDL) – Triglyceride (TAG) synthesis. Therefore, fructose can rapidly and without any control, produces glucose, glycogen, lactate, and pyruvate, providing both the glycerol and acyl portion for acyl-glycerol molecules. These particular substrates and the lack of regulation of this pathway could result in large amounts of TAG that can be packed into very-low density lipoproteins (VLDL) by the liver. Therefore, long term consumption of fruit nectars could cause health complications in human body altering the serum lipid parameters.

The fructose intake less than 50 g per day had no significant effect on fasting triacylglycerol level (18). Consumption of fructose more than the safe level (50 g per day) can rapidly induce insulin resistance, postprandial hypertriglyceridemia, and blood pressure in humans more than starch (or glucose). Moreover, it is a potential risk factor for fatty liver disease. Among the brands tested in the present study, consumption of a single bottle (200 ml) of either mango or mixed fruit nectar from brand A or E will fulfill 25% of the safe level of fructose.

According to research in Hong Kong (6), among the tested fruit juice products, 84.8 % types contained sucrose, 27.3% of contained glucose and 2.6% contained fructose as free sugar ingredients (no of products contain each free sugar /total no of products). However, all the selected fruit juice and nectar products used for the present study revealed the presence of free fructose and free glucose.

According to the Sri Lankan food preservative regulations in 1990, maximum permitted level of benzoic acid for fruit nectars and Ready to Serve (RTS) drinks fruit juices is 160 ppm. If a person consumes either brand B, D or H of mango nectar twice a day, will exceed the permitted level of benzoic acid. However

brand D mixed fruit nectar already exceeds the permitted level.

According to the new amendment of the preservative regulations for processed beverages (effective from 31st December 2020), no processed beverage except carbonated beverages should contain benzoic acid. Out of the 04 brands tested, 03 brands contained benzoic acid in nectar.

Some labels mislead the consumers indicating that the product contains no preservatives or any artificial flavors. Further, in some products, even with a significant amount of benzoic acid content, the manufacturers have not indicated the presence of benzoic acid on labels. Previous studies have pointed out that long term consumption of benzoic acid and its salt can cause many health problems and also affect body functions and metabolic processes where glycine is involved.

Moreover, storing the fruit nectar and juices exposed to sunlight, especially in retailed outlets can trigger chemical reactions giving rise to toxic derivatives (10). The observed significant difference in benzoic acid amounts in fruit nectar/ juices at supermarkets and retail outlets could be due to the synthesis of benzene and other products in the presence of vitamin C and sunlight. Benzene is a group 1 human carcinogen thereby, frequent consumption of fruit based products stored under sunlight can lead to many deleterious health hazards. However some of recent studies revealed, 0.5% of benzoic acid supplementation increased the digestibility of total energy and amino acids (28).

American Beverage Associations introduce some points of manufacturing process to suppress benzene formation. When acidity is low, ascorbic acid together with benzoic acid is very likely to produce benzene. Raw carbon, Coloring agents and flavors may contain ascorbates that consider removing, reducing, or replacing benzoates with other microbial growth inhibitors. Manufactures and traders advice to check the product storage conditions since strong light and high temperatures speed up the formation of free radicals and advised to avoid or minimize transition metals formation (29).

**5.Conclusions**

There was a difference in free fructose and free glucose contents in selected commercially available brands of mango and mixed fruit flavored fruit nectars in the Anuradhapura town area. Although fructose and glucose content per serving (200 ml) was within the safe level, 200 ml of mixed fruit nectar in two brands out of seven tested brands will fulfill 25% of the fructose safe level. Some brands of fruit nectars contained benzoic acid without indicating its presence on labels. Exposure to sunlight during storage markedly reduces the benzoic acid level eliciting the synthesis of other compounds from benzoic acid.

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