Determination of Preservatives in Fruit Juice Products Available in Bangladesh by a Validated RP-HPLC Method

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ABSTRACT: The aim of this study was to investigate whether fruit juices available in markets of Bangladesh contain any preservative. A specific RP-HPLC method was developed, validated and applied to identify and quantify preservatives including benzoic acid, sorbic acid, methyl paraben and propyl paraben simultaneously in 50 different products. These additives were separated by C_{18} column in mobile phase composed of methanol and acetate buffer (pH 4.4) in the ratio of 50:50 with a flow rate of 0.7 mL/min, and detected at 254 nm. Linearities for benzoic acid, sorbic acid, methyl paraben and propyl paraben were determined in the range of 20-170 ppm (r^2 0.997), 12-42 ppm (r^2 0.994), 10-60 ppm (r^2 0.993) and 10-60 ppm (r^2 0.992) respectively. Limit of detection (LOD) and limit of quantification (LOQ) were 5.46 ppm and 16.5 ppm for benzoic acid while for sorbic acid they were 1.08 ppm and 3.30 ppm, respectively. Benzoic acid was detected in a range of 96.1 to 441 ppm in 9 fruit juices while in 7 fruit juices sorbic acid was found in a range of 105 - 444 ppm. The values were within the maximum allowable ranges for fruit juice (1000 ppm for both benzoic acid and sorbic acid) as suggested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). None of the juice product was found to contain methyl paraben or propyl paraben.

Key words: Benzoic acid, sorbic acid, methyl paraben, propyl paraben, fruit juice, RP-HPLC.

INTRODUCTION

One of the major threats of food security is using preservatives in food products like fruit juices. According to Codex Alimentarius Commission, fruit juice is the unfermented but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means including post-harvest surface treatments.[1] In Bangladesh, there is no guideline about consumption of safe volume of fruit juices. Fresh fruit juice provides antioxidants, vitamins, nutrients as well as enzymes essential for digestion. The nutritional value and health curing effect of juices make it more and more popular among the consumers. On the other hand, marketed juices contain mainly water, sugar, preservatives, color and fruits pulps.[2] Foods when stored undergoes many chemical changes resulting in deteriorating its quality and nutritive value due to microorganisms, oxygen, or internal enzymatic development. To retain its qualitative values for a certain period of time during transportation, storage and consumption, it can be preserved by heating, cooling, refrigeration, freezing, air-proof packaging, drying, and fermentation.[3] However, sometimes these preservation techniques might not be suitable, hence food additives like preservatives can be added intentionally. Preservatives which are mostly used in different marketed fruit juices are benzoic acid (BA), sorbic acid (SA) and parabens. They are deliberately added to stop or delay nutritional losses due to microbiological, enzymatic or chemical changes and thus increasing its shelf life. They are very effective

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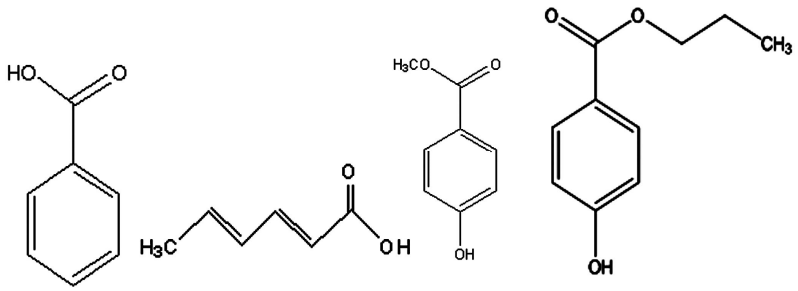
to control mold and inhibit yeast growth, and against a wide range of bacterial attack.[4] Although, preservatives are used mainly to prevent food from spoilage, excess amount of these can cause serious harmful effect such as headaches, palpitations, allergies, asthma and skin rashes.[5]

BA and its salts such as sodium benzoate, potassium benzoate are widely used as food preservatives.[6] They have inhibitory effects on the growth of yeast, a major cause of food spoilage. But due to the toxicity of BA its usage in foods should be controlled. Codex Alimentarius, an international treaty dictating food safety standards, limits the amount of BA or sodium benzoate to 0.05 to 0.1 percent by volume and allowed up to 1000 mg per kilogram for foods.[7] BA is responsible for asthma problems and increased levels of hyperactivity in children. If it is inhaled, it can cause damage to the nervous system. In infants and children, especially those with spastic paralysis or brain damage, it may be more likely to cause severe side effect.[8] It is also reported to cause obesity, diabetes, cancer in children.[9]

SA, is a natural organic compound, has been used as a food preservative since the 1940's. It is primarily used as an antifungal agent, but it also possesses antibacterial properties.[10] Though it is considered as nontoxic material, it has some adverse reactions like irritant skin reactions, allergic hypersensitivity skin reactions and perioral contact urticaria.[11]

For over 70 years, parabens have been used as preservatives in foods at concentrations of between 450 and 2000 ppm.[12] Because they possess certain properties such as broad antimicrobial spectrum activity, good stability, non-volatility and effectivity in a wide pH range, effective inhibitor of molds, yeasts and other microorganisms that commonly grow on food products.[11,13] But four most widely used parabens (namely methyl-, ethyl-, propyl-, and butylparaben) were found to be weakly estrogenic.[14,15] However, the European Union permits them as food additives with an acceptable daily intake (ADI) of 10 mg/kg bw/day.[16] Methylparaben (MP) and propylparaben (PP) are the most commonly used parabens and are often used together in 3:1 since they have synergistic effects.[3,17] It has been found that the antimicrobial activities of the parabens seem to increase with increasing chain length, but longer alkyl chains have limited application due to lower solubility.[15,18] Despite of having antimicrobial activities MP and PP interfere with the functioning of the endocrine system.[14] They are also associated with different health related issues of the infants and children such as developmental disorders, dysfunction of the immune system, learning problems as well as reproductive disorders.[19,20] Parabens are known to be estrogenic *in vitro* and estrogenicity appears to increase with side chain length.[21,22] In addition to this, parabens are also responsible for adversely interfering with the male reproductive system.[23]

Here, figure 1. represents the chemical structure of BA, SA, MP and PP respectively.



Benzoic Acid Sorbic Acid Methyl paraben Propyl Paraben

Figure 1. Chemical Structure of BA, SA, MP and PP.

Many analytical methods such as TLC,[24] capillary electrophoresis,[25,26] gas chromatography,[27-29] and spectrophotometry have been reported to determine preservatives. The most common analytical method for determination of BA, SA and parabens is RP- HPLC.[6,30-33] Since preservatives are widely used in consumer products, proper investigation of these are required for food safety and public health concern.

In continuation of our research work in the field of analysis of food, beverage[34] and dairy products[35,36] here, we report a simple, robust, economic and validated method for routine analysis of preservatives as well as to determine commonly used preservatives in marketed fruit juices available in Bangladesh.

MATERIALS AND METHODS

Sample collection. Fifty different commercial fruit juices of various flavor categorizing as mango, apple, orange, strawberry, pineapple, guava, litchi, grapes, coconuts and mixed fruits were purchased from different confectionaries, supermarkets and local markets in Dhaka city, Bangladesh during March-August, 2015. Among these samples 28 were domestic products whereas 22 were imported products. The list of collected juices are presented in table 1.

Table 1. List of different types of juice.

Type of Juice	Domestic	Imported	Quantity
Mango	17	3	20
Apple	2	4	6
Orange	2	1	3
Pineapple	2	2	4
Strawberry	1	4	5
Guava	1	2	3
Grape	1	2	3
Litchi	2	0	2
Coconut	0	1	1
Mixed fruit	0	3	3
Total	28	22	50

Chemicals and reagents. All the four standards benzoic acid, sorbic acid, methylparaben and propylparaben were gifted by Eskayef Bangladesh Limited, Gazipur, Bangladesh. To carry on the analysis, HPLC grade methanol (Fisher Scientific, India), acetic acid (Merck, India) and analytical grade ammonium acetate (Merck, Germany) were used.

Instrumentation. The analytical separation was carried out on HPLC system (Model LC-20 AT Shimadzu, Japan) equipped with UV/visible detector (Shimadzu SPD 20 A) and Degasser (Shimadzu DGU 20 A3) and connected with a computer. For the analyses, a C_{18} column (Capcell pak, 150 mm × 4.6 mm i.d., 5µm particle size) was used.

Chromatographic conditions. The mobile phase consisted of methanol and acetate buffer (pH 4.4) at a ratio of 50:50. The flow rate of mobile phase was 0.7 mL/min and the injection volume were 20 µl. The detection wavelength was set at 254 nm.

Preparation of standard and working solutions. Individual standard solution of each BA, SA, MP and PP were prepared at a conc. of 1000 ppm. Then, six standard solutions of each were prepared by diluting with mobile phase. Finally, standard solutions of BA in a range of 20-170 ppm, SA in a range of 12-42 ppm, MP and PP in a range of 10-60 ppm were prepared.

Preparation of sample. Accurately measured 40 mL of marketed product was taken in a beaker and it was diluted sufficiently by adding diluting solvent (mobile phase). Then 30 mL of sample was then taken into a centrifuge tube and centrifuged for 10 minutes at 4000 rpm. The supernatant was collected and filtered using a Whatman 41 filter paper to obtain the final sample.

Validation. Validation of the procedure was performed following pharmaceutical regulatory guidelines ICH Q2 (R1). A number of parameters such as system suitability, linearity, sensitivity, accuracy, precision, specificity and robustness were observed for this purpose.

System suitability. To assess the system suitability, repeatability, retention time, theoretical plate and tailing factor of six replicates of working standard solutions were used. The percentage relative standard deviation (%RSD) was calculated in each case.

Linearity. Standard solutions of BA, SA, MP, PP with their six different concentrations (ranging from 20-170 ppm for BA, 12-42 ppm for SA, 10-60 ppm for both MP and PP) were prepared and analyzed in triplicate to prove the linearity of system. Calibration curves were made using MS Excel 2007 for each standard component.

Sensitivity. The limit of detection (LOD) is defined as the smallest peak detected with a signal height three times that of the baseline; while the limit of quantitation (LOQ) refers to the lowest level of analyte which can be determined with an acceptable degree of confidence. LOQ value is often calculated as 10 times the signal height to the baseline. LOD and LOQ were calculated in accordance with the 3.3 s/m and 10 s/m criteria, respectively, according to ICH Q2 (R1) recommendations, where 's' is the standard deviation of the peak area and 'm' is the slope of the calibration curve, determined from linearity investigation.

Accuracy (Recovery test). For Recovery, test was done by analyzing six replicates of a sample of known concentration of standard solutions. Then percent recoveries (mean ± %RSD of six replicates) were calculated.

Precision. Intra-day precision was determined from standard solution and sample by injecting 20 µl. %RSD was calculated for six replicates of standard and sample solution. For inter-day precision, sample solution was carried out by another analyst daily for six times over a period of three days and %RSD was calculated.

Specificity. The chromatograms of blank injection, standard injection and test sample injection used to justify the specificity of target analytes.

Robustness. To determine the robustness of the method, the effect of change in wavelength was studied at 252 and 256 nm instead of 254 nm. Also, the flow rate was changed to 0.6 and 0.8 ml/min instead of 0.7 ml/min. The mobile composition was studied at (Buffer: Methanol = 52:48) and (Buffer: Methanol = 48: 52) ratio instead of (Buffer: Methanol = 50: 50) ratio. The % RSD for each case was calculated.

Ruggedness. Ruggedness of the method was determined by analyzing six assay sample solutions of standards by two analysts in the same laboratory to check the reproducibility of the result. The percentage recovery and %RSD were calculated in both cases.

RESULTS AND DISCUSSION

Method validation

System suitability. The system suitability test (SST) was performed during validation procedure. SST parameters including peak area, retention time, column efficiency (number of theoretical plates, (N) and tailing factor (T) listed in table 2 was established by six replicates of standard solution containing benzoic acid, sorbic acid, methyl paraben and propyl paraben, respectively. The %RSD values for the calculated SST parameters for 6 replicates were less than 2% which meets the acceptance criteria according to ICH guidelines.

Linearity. The regression equations were calculated as $Y = A + BX$, where Y is peak area and

X is the concentration in ppm of the standard solutions of mixture of four standards. The correlation coefficients (r^2) of the respective standard solutions in the prescribed ranges were 0.997 (20-170 ppm of BA), 0.994 (12-42 ppm of SA), 0.993 (10-60 ppm of MP) and 0.992 (10-60 ppm of PP) as shown in table 3.

Sensitivity. The LOD and LOQ were determined using calibration curve method according to ICH Q2 (R1) recommendation. The corresponding data is in table 4.

Accuracy. The validity and reliability of proposed method was assessed by recovery studies by standard addition method. Results of studies are given in table 5 and 6. From table 6, it was observed that the recovery (%) of the analytes of the standard solution were within 98-100% which resembles an accurate method. The observed the recovery (%) of the four analytes from the sample were within 100% which indicates the accuracy of the proposed method as shown in table 6.

Precision. Intra-day precision of four analytes were investigated for both standard and sample solutions. In case of inter-day precision, the sample was carried out by a second analyst in the second day. From tables 7 and 8, it was apparent that the RSD (%) of peak area and assay were found to be less than 2% which indicated that the method was precise.

Robustness. The %RSD of robustness testing under different conditions is shown in table 9, which indicates that the proposed method was robust.

Specificity. Peaks were identified by comparing with the retention times of standards and confirmed the characteristics spectra in both sample and standard solution. Chromatograms of blank, standard solutions and a sample are shown in the figure 2.

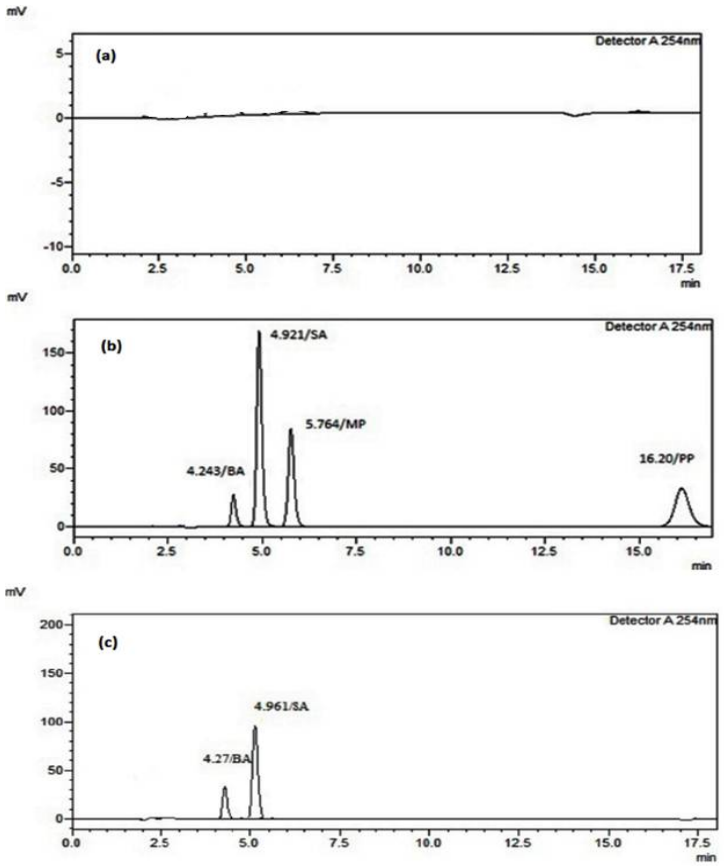


Figure 2. Chromatograms of blank (a), standard solution (b) and a sample (c).

Table 2. System suitability data of BA, SA, MP and PP.

Analytes	Peak area (mean± %RSD)*	Retention time (min) (mean ± %RSD)*	Theoretical plate (mean± %RSD)*	Tailing factor (mean± %RSD)*
Benzoic acid	84,376 ± 0.945	4.25 ± 0.24	5153 ± 1.30	1.210 ± 0.48
Sorbic acid	646,761 ± 0.95	4.90 ± 0.20	5838 ± 1.33	1.194 ± 0.19
Methyl paraben	482,735 ± 0.78	5.59 ± 0.30	6175 ± 0.661	1.169 ± 0.17
Propyl paraben	201,895 ± 0.82	15.60 ± 0.50	8438 ± 0.39	1.105 ± 0.39

*n= 6 replicates

Table 3. Linearity and regression analysis of BA, SA, MP and PP.

Analytes	Concentration (ppm)	Peak area (mean ± % RSD)*	Statistics
Benzoic acid	20	56489 ± 1.2	Regression correlation coefficient, (r^2) = 0.997
	50	72355 ± 1.03	
	80	84543 ± 0.94	y-intercept = 46781
	110	98730 ± 1.33	
	140	112730 ± 0.954	Slope of regression line = 482.1
	170	130687 ± 1.31	
Sorbic acid	12	448816 ± 1.43	Regression correlation coefficient, (r^2) = 0.994
	18	544180 ± 1.11	
	24	646761 ± 0.95	y-intercept = 21163
	30	776815 ± 1.01	
	36	862935 ± 0.98	Slope of regression line = 18676
	42	1015924 ± 1.27	
Methyl paraben	10	334858 ± 1.45	Regression correlation coefficient, (r^2) = 0.992
	20	408190 ± 1.07	
	30	481068 ± 0.86	y-intercept = 227793
	40	576899 ± 0.98	
	50	698536 ± 1.4	Slope of regression line = 9102
	60	778622 ± 1.03	
Propyl paraben	10	145989 ± 0.97	Regression correlation coefficient, (r^2) = 0.993
	20	177663 ± 0.99	
	30	202393 ± 0.89	y-intercept = 10673
	40	239985 ± 1.07	
	50	275894 ± 1.54	Slope of regression line = 3436
	60	338067 ± 1.22	

*n=6 replicates

Table 4. LOD and LOQ of standards BA, SA, MP and PP.

Component	Lower limit of detection (ppm)	Lower limit of quantification (ppm)
Benzoic acid	5.46	16.5
Sorbic acid	1.08	3.30
Methyl paraben	3.65	10
Propyl paraben	1.60	4.85

Table 5. Recovery of standard solutions of BA, SA, MP and PP.

Analytes	Actual concentration (ppm)	Calculated concentration (ppm)	(Mean recovery ± %RSD)*
Benzoic acid	50	50.05	99.82 ± 0.6
		49.92	
		49.80	
	80	80.10	100.07 ± 0.5
		80.08	
		79.97	
	110	111.81	100.07 ± 1.5
		108.78	
		109.67	
Sorbic acid	18	18.24	100.453 ± 0.6
		17.96	
		18.05	
	24	23.97	100.226 ± 0.25
		24.09	
		24.14	
	30	29.63	99.69 ± 0.76
		29.91	
		30.20	
Methyl paraben	20	20.40	100.61 ± 1.04
		19.89	
		20.08	
	30	30.07	100.19 ± 0.38
		30.20	
		29.93	
	40	39.87	99.92 ± 0.16
		40.06	
		39.98	
Propyl paraben	20	19.70	98.7 ± 0.57
		19.91	
		19.62	
	30	30.05	99.85 ± 0.24
		29.87	
		29.95	
	40	40.32	99.9 ± 0.63
		39.76	
		39.83	

Table 6. Recovery of sample solution of BA, SA, MP and PP.

Analytes	Actual concentration (ppm)	Added concentration (ppm)	Calculated concentration (ppm)	% recovery (mean ± %RSD)*
Benzoic acid	26.08	50	75.50 75.15 75.91	98.64 ± 0.64
		80	106.00 106.10 105.36	98.00 ± 0.90
		110	135.06 135.78 134.39	98.33 ± 0.47
Sorbic acid	28.24	18	45.91 46.02 46.10	98.71 ± 0.47
		24	52.10 51.93 52.01	99.07 ± 0.26
		30	57.95 57.80 57.86	98.56 ± 0.42
Methyl paraben	10	20	29.75 29.03 29.59	98.99 ± 0.33
		30	39.61 39.15 39.92	98.65 ± 0.75
		40	49.05 49.84 49.61	98.33 ± 0.58
Propyl paraben	10	20	29.66 29.85 29.19	98.00 ± 0.75
		30	39.04 39.52 39.69	98.35 ± 0.63
		40	48.99 48.11 48.63	98.02 ± 1.12

Table 7. Intra-day precision of BA, SA, MP and PP.

Analytes	Standard solution		Sample M5	
	Peak area (mean ± %RSD)*	Assay (ppm) (mean ± %RSD)*	Peak area (mean ± %RSD)*	Assay (ppm) (mean ± %RSD)*
Benzoic acid	84,376 ± 0.95	77.99 ± 1.93	59,022 ± 0.2	25.39 ± 1.29
Sorbic acid	646,761 ± 0.95	33.48 ± 0.87	547,606 ± 0.5	28.19 ± 0.46
Methyl paraben	482,735 ± 0.78	27.48 ± 1.39	310,872 ± 1.2	9.16 ± 1.6
Propyl paraben	201,895 ± 0.82	28.54 ± 1.02	135,989 ± 1.4	9.59 ± 1.4

*n=6 replicates

Table 8. Inter-day precision of BA, SA, MP and PP.

Analytes	Analyst-1		Analyst -2	
	Peak area (mean ± %RSD)	Assay (ppm) (mean ± %RSD)	Peak area (mean ± %RSD)	Assay (ppm) (mean ± %RSD)
Benzoic acid	59022 ± 0.2	25.39 ± 1.29	59330 ± 1.3	25.72 ± 1.16
Sorbic acid	547606 ± 0.5	28.19 ± 0.46	540516 ± 1.25	28.08 ± 0.08
Methyl Paraben	310872 ± 1.2	9.16 ± 1.6	310753 ± 0.98	9.02 ± 1.8
Propyl Paraben	135989 ± 1.4	9.59 ± 1.4	145026 ± 0.64	9.8 ± 1.5

*n=6 replicates

Table 9. Robustness of method.

Component	Parameter	Changed condition	Amount of standard solution (ug/ml)	Amount of standard solution detected (mean ± %RSD)*
Benzoic Acid	Change in wavelength (nm)	252	80	79. 93 ± 1.3
		254	80	80.05 ± 0.05
		256	80	79.88 ± 1.43
	Acetate buffer: methanol	52:48	80	79.90 ± 1.44
		50:50	80	80.05 ± 0.05
		48:52	80	80.03 ± 1.18
	Change in flow rate (mL/min)	0.6	80	79.97 ± 1.11
		0.7	80	80.05 ± 0.05
		0.8	80	80.08 ± 1.44
Sorbic acid	Change in wavelength (nm)	252	24	23.98 ± 1.43
		254	24	24.06 ± 0.25
		256	24	24.02 ± 1.39
	Acetate buffer: methanol	52:48	24	23.88 ± 1.11
		50:50	24	24.06 ± 0.25
		48:52	24	23.97 ± 1.17
	Change in flow rate (mL/min)	0.6	24	23.99 ± 0.88
		0.7	24	24.06 ± 0.25
		0.8	24	24.10 ± 1.42
Methyl paraben	Change in wavelength (nm)	252	30	30.09 ± 1.45
		254	30	30.22 ± 0.66
		256	30	30.20 ± 0.86
	Acetate buffer: methanol	52:48	30	30.24 ± 0.85
		50:50	30	30.22 ± 0.66
		48:52	30	30.30 ± 1.44
	Change in flow rate (mL/min)	0.6	30	29.99 ± 1.11
		0.7	30	30.22 ± 0.66
		0.8	30	30.01 ± 1.52
Propyl paraben	Change in wavelength (nm)	252	30	29.09 ± 1.45
		254	30	29.95 ± 0.89
		256	30	30.03 ± 0.77
	Acetate buffer: methanol	52:48	30	29.70 ± 1.12
		50:50	30	29.95 ± 0.89
		48:52	30	29.88 ± 1.02
	Change in flow rate (mL/min)	0.6	30	29.97 ± 1.54
		0.7	30	29.95 ± 0.89
		0.8	30	29.90 ± 0.98

*n=3 replicates

Quantitation of marketed fruit juices. The aim of this study was to investigate and quantify commonly used preservatives in marketed fruit juices. The validated RP-HPLC method was used to carry out the analysis which results in simultaneous detection of BA, SA, MP and PP approximately within 4.25, 4.9, 5.59 and 15.6 minutes, respectively.

For this purpose, 50 different fruit juices were collected and after analysis some of the samples showed sharp peak on the position of BA and SA in the chromatogram (figure 2). Some fruit juices products also showed small peaks on the position of BA, SA, MP and PP but were not considered in quantitation as these were below the LOQ. It was observed that only 11 juices were found to contain BA or SA either alone or in combination. Methylparaben and propylparaben were not found in any of the samples.

Among the 28 domestic products 9 juices were found to contain benzoic acid or sodium benzoate either alone or in combination with sorbic acid and 7 juices were found to contain sorbic acid or combination with benzoic acid. About 50% (5 out of 11) of the positive samples were found to contain mixture of benzoic acid and sorbic acid. But none of the imported 22 juices were found to contain preservatives. The total scenario of the obtained results is shown in table 10

Although 6 juices were label claimed to contain benzoic acid, after analysis 9 juices were found to be benzoic acid positive juice. In case of sorbic acid 3 juices were label claimed, whereas 7 were found to contain sorbic acid. The juice products analyzed, didn't mention the amount of preservatives used in label.

From our study we found that about 35% (7 out of 20) mango juices contain benzoic acid, 15% (1 out of 3) orange juices contain benzoic acid and 50% litchi juices (1 out of 2) contain benzoic acid. In case of sorbic acid, the scenario is about 20% (4 out of 20) mango juices contain sorbic acid, 15% (1 out of 3) orange juices contain sorbic acid and 100% litchi juices (2 out of 2) contain sorbic acid. According to JECFA, acceptable daily intake (ADI) value of benzoic and sorbic acid is 1000 ppm. From the analysis it is apparent that none of the preservatives positive juices exceed the allowable limit. Figure 3, 4 and 5 represent the brief overview of investigated BA and SA containing products, respectively.

Similar studies had been conducted in other countries like Brazil, Australia, USA, Malayasia, China and Portugal. Tfouni *et al*[24] found BA and SA at a mean conc. of 495 and 51 ppm in fruit juices from China on their research on ″Determination of benzoic and sorbic acid in Brazilian food″. Sorbic acid content (105-445 ppm) that we found during research works was greater than this reported content (51 ppm). However, in case of Portugal the amount of SA and BA in fruit juices were found 210±5.2 ppm and 153±1.1 ppm, which is almost same as ours investigated results. Tang *et al*[37] on their study ″A quick method for the simultaneous determination of ascorbic acid and sorbic acid in fruit juices by capillary zone electrophoresis″ found no sorbic acid in orange juices whereas in our study we found 3 orange juices containing BA and SA.

So, the overall finding from the analysis is none of the juices were in violation with rules set by JECFA. But one notable thing is that some fruit juices contain preservatives, but they are not label claimed. Again, amounts of additives are not mentioned on the label. Consumers of fruit juices are mostly school going children. Intaking fruit juices means taking preservatives as well. However, many children may intake fruit juices multiple times a day that indicate intaking preservatives beyond the permittable limit. So, there should have clear instructions for maximum safe volume of juices along with preservative's name and amount in the label of each juice container. For example, according to this research, considering ADI value for benzoic acid is 5 mg/kg weight as well as assuming the weight of a child as 30 kg and weight of adult 70kg, the maximum safe volume of juice for consumption is calculated (table 11).

Table 10. BA and SA contents found in marketed fruit juices.

	Number of sample	Label claim for BA	BA positive	BA content found (ppm)	BA>1000 ppm*	Label claim for SA	SA positive	SA content found (ppm)	SA>1000 ppm*
On the basis of sources									
Domestic juices	28	6	9	96-467	-	3	7	105-445	-
Imported	22	-	-	-	-	-	-	-	-
On the basis of types									
Mango	20	6	7	96-288	-	2	4	136-445	-
Orange	3	-	1	467	-	-	1	444	-
Litchi	2	-	1	234	-	1	2	105-186	-

*Codex general standard for food additives set by JECFA

Table 11. Safe volume of benzoic acid containing juice for consumption.

Code of juice	Maximum safe volume (L) (approximate) for children	Maximum safe volume (L) (approximate) for adult
M5	0.57	1.34
M11	0.64	1.5
M12	0.71	1.6
M17	1.5	3.6
M14	1.3	3.48
M21	0.51	1.2
M22	1.41	3.21
M41	0.414	0.79
M42	0.32	0.96

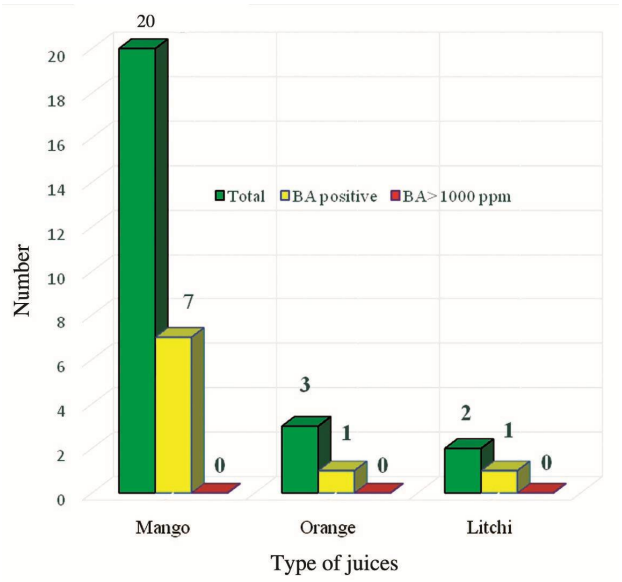


Figure 3. Overview of BA containing juices

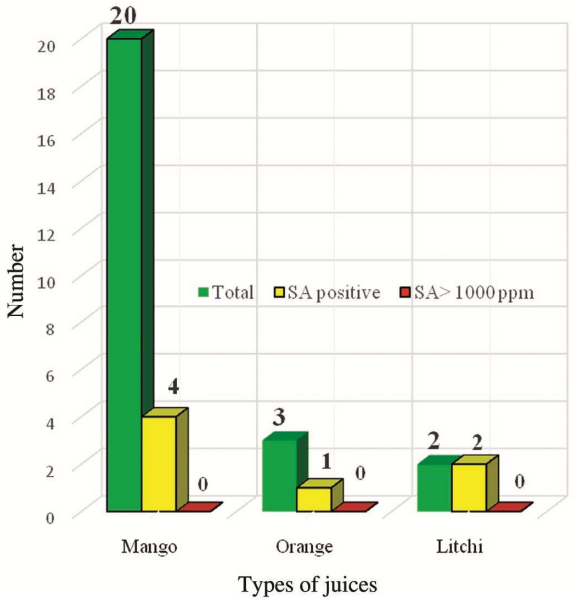


Figure 4. Overview of sorbic acid (SA) containing juices according to types.

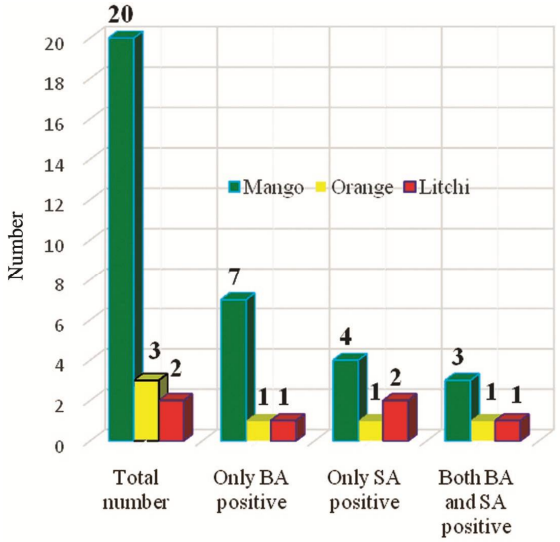


Figure 5. Overview of benzoic acid and sorbic acid containing juices according to type.

Table 12. Safe volume of sorbic acid containing juice for consumption.

Code of juice	Maximum safe volume (L) (approximate) for children	Maximum safe volume (L) (approximate) for adult
M5	2.6	6.19
M11	4	9.4
M20	5.5	12.8
M21	7.0	16.6
M27	6.8	15.88
M41	1.6	3.9
M42	1.6	3.9

`Similarly, for sorbic acid containing juice- considering ADI value for sorbic acid is 25 mg/kg weight and assuming the weight of a child as 30 kg and weight of adult 70 kg the maximum safe volume of juice for consumption is calculated (table 12).

So, the regulatory authorities should put emphasis on setting rules and regulation regarding this issue to ensure public health protection.

CONCLUSION

At present, fruit juices have become a favorite drink both for children and adults. But uses of preservatives in juices have become major threat for human health as they can cause different life-threatening diseases. So, our main target was to check the marketed fruit juices to verify whether they contain any preservatives as well as their contents they used. From this study the scenario of using preservatives in fruit juices in perspective of Bangladesh has been revealed. We found that among the 50 marketed fruit juices, 11 were contained preservatives (BA and SA). As fruit juices are highly consumed by adolescents especially by the school going children, an excess amount of preservatives can cause many serious health problems. One of them is behavioral change especially in children. Besides, another most serious harmful effect of preservatives is their ability to transform into carcinogen when digested. So, too much use of preservatives should be prohibited. However, our suggestion is that marketed fruit juices should be investigated by the concerned authorities as well as independent research groups at regular interval across the country. In such case, this validated RP-HPLC method can be used for the routine analysis of fruit juices. Finally, the rules and regulations regarding the usage of preservatives in fruit juices should be strictly imposed and practiced.

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