

## Levels of Benzoic and Sorbic Acid Preservatives in Commercially Produced Ready to Serve Products in Sri Lanka

**Krishanthi Karunarathne\***

Senior Assistant Government Analyst, Government Analyst Department, Sri Lanka

\*Corresponding Author: Krishanthi Karunarathne, Senior Assistant Government Analyst, Government Analyst Department, Sri Lanka.

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### Abstract

Benzoic acid, sorbic acid are widely used for food preservation. The analyses of these preservatives in 100 different food products were performed. Therefore 100 samples including, 10 carbonated beverages, 20 jams, 10 jelly drinks, 10 jelly cups, 30 ready to serve drinks, 10 nectars, and 10 sauces were analysed by using high performance liquid chromatography. The HPLC determination of the preservatives was performed using a reversed – phase C18 column and UV detection at 235 nm. Flow rate approximately 1.2ml/min. Eluent for HPLC, mix 50 volume parts of Ammonium Acetate solution with 40 volume parts of methanol for HPLC and adjust to a pH of 4.5 to 4.6 with acetic acid. The preservative concentration in samples was used as external standards for sodium benzoate and potassium benzoate. Levels were compared with Food Act and Food Standards Regulation in Sri Lanka. Both benzoic acid and sorbic acid were detected in a range of 1120ppm to 30ppm and 582ppm to 16ppm respectively. Although 65% of the samples was not compliant with the Food Act and Food Regulations in Sri Lanka.

**Keywords:** Benzoic Acid; Sorbic Acid; Sri Lanka

### Introduction

According to Sri Lanka Regulations preservatives are chemical substances which when added to food is capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food. Examples of preservatives which permitted specified in Sri Lanka. Benzoic acid, Sodium benzoate, Potassium benzoate, Calcium benzoate mainly

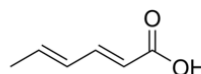
- Methy 4 – hydroxyl benzoate
- Methyl 4 – hydroxyl benzoate Sodium salt
- Methy 4 –hydroxyl benzoate
- Ethyl 4 – hydroxyl benzoate Sodium salt
- Propyl 4 – hydroxyl benzoate
- Propyl 4- hydroxyl benzoate Sodium salt
- Sodium nitrate
- Potassium nitrate
- Sodium nitrite
- Potassium nitrite
- Propionic acid
- Sodium propionate
- Calcium propionate

Another main one Sorbic acid

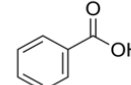
- Sodium sorbate,
- Potassium sorbate,
- Calcium sorbate and Sulphur dioxide Sodium sulphite
- Sodium hydrogen sulphite
- Sodium metabisulphite
- Potassium metabisulphite
- Calcium sulphite
- Calcium hydrogen sulphite
- Biphenyl, 2-Hydroxybiphenyl, Hexamine, Nisin.

In this study as preservatives we were used only Benzoic Acid and Sorbic acid.

Structure of Sorbic Acid (E 200)



Structure of Benzoic Acid (E 210)



Figure

Preservatives are chemical substances which are added to the food to prolong its shelf – life. Inhibiting the bacterial and fungal growth as they lag the spoilage accident. Several studies proved the mutagenicity of Sorbic and Benzoic acids. Induce mutations in human DNA (therefore their determinations in food product is a must), Intestinal upset, Allergies, Skin rashes, Headaches, Hyperactivity (already affected by children).

The long term effects also affected. Cancer induction (like bladder cancer), Respiratory problems, For these reasons the use of food additives in different countries is limited by this specific regulations. These preservatives are allowed by legislation but their use demands special care.

Like other countries the use of these preservatives in Sri Lanka also have been restricted. In Sri Lanka followed Food Act and Food Standards Regulations prepared by Food Control Administration Unit of the ministry of healthcare and nutrition in Collaboration with the World health Organization. And SLS – Sri Lanka Standards (specifications for food preservatives). This Sri Lanka Standard was authorized for adoption and publication by the Council of the Sri Lanka Standards institution on 1990 – 08 – 13. After the draft finalized by the Drafting committee on food products Divisional Committee.

The aim of this study was to determine the Sorbic and Benzoic acids in different food products. By using RP – HPLC apparatus.

**Such foods are**

- 10 Carbonated Beverages
- 20 Jams
- 10 Jelly drinks
- 10 jelly cups
- 10 Nectars
- 10 Sauces
- 30 Ready to Serve drinks

Because of the huge consumption of these different food products, especially by children, which intrinsically rely on the usage of these products.

Therefore there is a dire need to determine their concentrations within different food products in order to guarantee that they are within the normal ranges stated by Food Act and Food Standards Regulations in Sri Lanka.

Product	Food Act and Regulation Ranges for Sorbic Acids (mg/kg)	Food Act and Regulation Ranges for Benzoic acid mg/kg
Carbonated Beverages	300	160
Nectars	300	160
Jelly Drinks	300	160
Ready to Serve Drinks	300	160
Sauces	1000	250
Jelly Cups	Not permitted	Not permitted
Jam	350	Not permitted

**Table 1:** Clarifies the permitted ranges for sorbic and benzoic acids in above different food commodities (According to FOOD ACT and REGULATIONS in Sri Lanka).

Many methods have been validated for the Benzoic and Sorbic acids determination purpose in different food products and commodities. The proposed ISO 22855: 2008 method for determination of Benzoic and Sorbic acid Fruit and Vegetable products enables the simultaneous and selectivity for analysis. High Performance Liquid Chromatography (HPLC) has high specificity, minimum sample preparation, Does not require derivatization.

**Materials and Methods**

Chromatographic analysis was carried out using High Performance Liquid Chromatography Agilent 1200 series

Equipped with

- Quaternary pump
- Vacuum degasser
- UV detector
- Analytical column, (Supelco C18 5 m x 250 x 4.6 mm)

**Principle**

Benzoic and Sorbic acid are extracted from test portion using a mixture of Ammonium acetate buffer solution and methanol, under acidic condition. The concentration of Benzoic and Sorbic acid is determined by means of HPLC using a reverse phase column and ultraviolet (UV) detector.

**Reagents and Materials**

- Acetic acid (CH3COOH) glacial
- Methanol (CH3OH) for HPLC
- Ammonium Acetate CH3COONH4, 0.01 mol/l solution
- Ammonium acetate/Acetic acid (CH3COONH4/CH3COOH) buffer solution.
- Sodium Benzoate and Sorbic acid stock solutions
- K4[Fe(CN)6].3H2O and ZnSO4.7H2O

**Extraction Solution**

Mix 60 volume parts of Ammonium Acetate/Acetic acids buffer solution with 40 volume parts of methanol.

**Eluent for HPLC**

Mix 50 volume parts of ammonium acetate solution with 40 volume parts of methanol for HPLC and adjust to a pH of 4.5 to 4.6 with Acetic acid. Filter the eluent over membrane filter.

**Sample collection**

The highly consumed ready to serve products with different brands in Sri Lanka were received from public health inspectors in Health Department. A total number of 100 samples were collected, to be representative of what a highly consumer in Sri Lanka.

**Preparation of test solution**

Homogenize or mix the sample carefully, Concentrated juice should be diluted to single strength. Clear Samples, Dilute 5.00g to 10.00g of a sample in approximately 75ml of extraction solution in a 100ml volume flask – ultrasonic bath at least 10 mins – Dilute to the mark. Filter through a membrane syringe filters 0.45 m.

**For cloudy samples**

All the procedure followed up equal to the clear sample. In addition, Have to add

- 1ml of K4[Fe(CN)6].3H2O
- 1ml of ZnSO4. 7H2O

**Procedure preparation of the calibration curves**

Dilute Benzoic Acid 1000mg/l and Sorbic Acid 1000mg/l stock solutions with extraction solutions. To obtain the standard mixture solutions. Concentrations of 5mg/l to 150mg/l respectively. Inject 10 l of each of the calibrations solutions into the chromatograph under the following conditions. Flow rate 1.2ml/min. Wavelength for UV detection 235nm. Prepare the calibrations curves by plotting the peak areas against BA and SA concentration in mg per litre.

**Determination**

Inject 10µl of the test solutions into the chromatograph using the same conditions as for the preparation of the calibration graph. Calculation for determination the concentration of BA and/or SA of

the sample CS in mg per liter, using the following equation. Concentration of BA or SA in the test sample (mg/kg), Cs

$$Cs = \frac{Xb/Xs \times 100 \times 1000}{1000 \times Ws}$$

Where, Xb is concentration of BA in the test solution obtained directly from the calibration curve. Ws is the test samples in grams.

**Validation of analytical method**

The analytical method was assessed for linearity, recovery, precision, and limit of detection before sample analysis.

**Result and Discussions**

Product	Sorbic acid (mg/kg)	Benzoic acid (mg/kg)
Rsd 1	nd	318
Rsd 2	nd	277
Rsd 3	nd	140
Rsd 4	78	478
Rsd 5	33	120
Rsd 6	102	151
Rsd 7	nd	375
Rsd 8	nd	169
Rsd 9	nd	419
Rsd 10	nd	158
Rsd 11	142	145
Rsd 12	nd	374
Rsd 13	nd	585
Rsd 14	nd	368
Rsd 15	nd	175
Rsd 16	nd	43
Rsd 17	23	138
Rsd 18	nd	390
Rsd 19	nd	212
Rsd 20	nd	55
Rsd 21	46	42
Rsd 22	nd	113
Rsd 23	60	318
Rsd 24	80	86
Rsd 25	nd	322
Rsd 26	nd	352
Rsd 27	23	138
Rsd 28	nd	631
Rsd 29	46	42
Rsd 30	nd	432

**Table 2:** Preservatives content in ready to serve drinks.

Product	Sorbic Acid (mg/kg)	Benzoic Acid (mg/kg)
J 1	nd	52
J 2	nd	446
J 3	nd	451
J 4	nd	146
J 5	nd	345
J 6	nd	49
J 7	nd	81
J 8	66	259
J 9	nd	584
J 10	nd	30
J 11	nd	81
J 12	nd	769
J 13	nd	575
J 14	nd	664
J 15	nd	368
J 16	nd	65
J 17	nd	55
J 18	nd	44
J 19	63	58
J 20	nd	866

**Table 3:** Preservatives content in Jam.

Product	Sorbic Acid(mg/kg)	Benzoic Acid(mg/kg)
N1	59	nd
N2	192	45
N3	nd	51
N4	31	nd
N5	nd	83
N6	nd	44
N7	36	40
N8	nd	164
N9	nd	184
N10	nd	288

**Table 4:** Preservatives content in Nectars.

Product	Sorbic Acid Content (mg/kg)	Benzoic Acid Content(mg/kg)
CB 1	165	nd
CB 2	nd	130
CB 3	nd	118
CB 4	69	190
CB 5	nd	194
CB 6	nd	219
CB 7	nd	199
CB 8	nd	225
CB 9	nd	126
CB 10	nd	219

**Table 5:** Preservatives Content in Carbonated Beverages.

Product	Sorbic Acid Content(mg/kg)	Benzoic Acid Content(mg/kg)
JC 1	149	nd
JC 2	84	219
JC 3	238	nd
JC 4	192	155
JC 5	184	52
JC 6	16	44
JC 7	292	nd
JC 8	333	380
JC 9	314	444
JC 10	30	211

**Table 6:** Preservatives Content in Jelly Cups.

Product	Sorbic Acid Content (mg/kg)	Benzoic Acid Content (mg/kg)
Jd 1	90	nd
Jd 2	nd	nd
Jd 3	215	297
Jd 4	215	nd
Jd 5	182	nd
Jd 6	nd	239
Jd 7	582	192
Jd 8	142	nd
Jd 9	90	59
Jd 10	120	nd

**Table 7:** Preservative content in Jelly Drink.

Product	Sorbic acid (mg/kg)	Benzoic Acid (mg/kg)
SA 1	nd	337
SA 2	nd	338
SA 3	nd	75
SA 4	nd	178
SA 5	nd	330
SA 6	164	214
SA 7	nd	1120
SA 8	204	172
SA 9	nd	205
SA 10	nd	422

**Table 8:** Preservative content in Sauces.

In table 2 to 8 shown the different content of Benzoic Acid and Sorbic Acid in all the 100 samples of carbonated Beverages, jams, jelly drinks, jelly cups, nectars, sauces, and ready to serve drinks. The each level of the preservatives tested were in compliance with the Food Act and Food Standards Regulations in Sri Lanka.

According to Sri Lanka Standard 729:2010 ready to serve drinks means A fruit drink intended for consumption without dilution and prepared from unfermented but fermentable fruit juice/puree/concentrate with or without some of the pulp and containing any soluble sweetener and portable water. For ready to serve drinks the legal limits of Benzoic acid was found to be 160mg/kg and sorbic acid 300mg/kg. In ready to serve drinks the range of Sorbic Acid content (19-478) mg/kg and Benzoic acid range (42-318) mg/kg. Out of 30 samples Sorbic Acid was not detected in 20 samples and Benzoic Acid detected in all the However in product 6 the concentration of BA is 151 and percentage of BA is 94% and SSA 34%. This sample also not compliance with the Food Standard Regulations in 1989. According to Food Preservatives Regulations 1989 if product contained combination of preservatives the total percentage of those preservatives shall not exceed hundred. According to the legal requirements, from them out of 30 samples 18 samples are violate.

According the SLS (265:1985) jams means the wholesome product obtained by processing to a suitable consistency, fruit ingredients which may be all fruit, fruit pulp or fruit puree, with water and a carbohydrate sweetner. According to schedule ii of Food Preservatives Regulations 1989, of Food Act and Regulations of Sri

Lanka Benzoic Acid is not a permitted preservatives in jam. But Sorbic Acid allowed upto 350mg/kg. The results of the analyses of the jam are collected in Table 3. In all the samples Sorbic Acid was not detected. Benzoic Acid detected in the range of (30-866) mg/kg. However the Benzoic Acid detected in all the samples. Out of 20 samples all the samples were poor quality.

According to SLS 1328: 2008 fruit Nectar means the unfermented but fermentable product obtained by adding water with or without the addition of sugars honey treacle and or syrups and or non nutritive sweetners to product given in fruit juices or mixture of those products. In the majority of the samples sorbic acid was detected in the range of (31-192) mg/kg and it was not detected in 6 samples. The Benzoic Acid was detected in the range of (40-288) mg/kg and not detected in 2 samples. Another thing according to SLS Requirements (SLS 1328:2008) nectar shall not contain any added artificial colours. According to Table 4 10 samples were tested, From these, 3 samples were contained benzoic acid that above the legal limits of 160mg/kg.

From SLS (183:1997) carbonated beverages means ready to drink nonalcoholic beverages prepared from portable water one or more of the ingredients given in sweetening ingredients, fruit juices, flavouring agents, like this saturated with carbon dioxide and packed in hermitically sealed containers. These may be plain, flavoured or sweetened. 10 samples were tested In table 5 is shown the average content of BA and SA in all the samples. For carbonated Beverages the legal limits of BA was found to be 160mg/kg and Sorbic Acid 300mg/kg. According to these 6 samples were not compliance of the Food Act. According to results of the range of BA (126-225)mg/kg. But Sorbic Acid was detected in two samples however the BA detected in 9 samples. Out of 10 samples 6 samples were not agree for the Standard Regulations and Act in 1989.

According to SLS standards jelly means the product prepared from a suitable fruit ingredients from which the insoluble solids have been removed during the process of manufacture and mixed with a carbohydrate sweetner with or without water and processed to a semi solid consistency. According to schedule ii of Food (preservatives) Regulations 1989 jelly (jelatine base sugar confectionery) is not a specified food article which may contain permitted preservatives. In table 6 shown the results of the analyses of jelly samples, the relavant range of Sorbic Acid (16-333) mg/kg and Benzoic Acid (52-444)mg/kg However the Sa and BA are accidentally exceeded,

which does not allowed which stated in Food Standard Regulations. All the samples were poor quality.

Actually for jelly drinks the legal limits of BA was found to be 160mg/kg and SA 300mg/kg. The results of the analyses of jelly drinks samples were collected in Table 7. The SA was detected in all the samples in the. of 90-582mg/kg. Additionally the concentration of BA was found in most of the samples contained in the range of 59-297mg/kg.

According to schedule ii of Food (Preservatives) Regulation 1989, for soft drinks, further if product contain more than one preservative sum of the percentage of those preservatives shall not exceed 100. But in jelly drink product 3 the concentration of BA is 297, percentage BA 185, and for SA 215, concentration SA 72 percentage. This sample also not compliance with the Food Standards Regulation in 1989. In jelly drinks also 3 samples were not compliance of the Food Act and Regulations.

For sauces we are analyzed in 3 kinds of sauces Tomato sauces, Chilli sauces, Soya sauces. According to schedule ii of Food (Preservatives) Regulation 1989 sauces shall not contain more than 250mg/kg BA and 1000mg/kg SA. The results of the analyses of the sauce samples were collected in Table 8. The BA was detected in all the samples in the range of (75 to 1120) mg/kg, while the SA was not detected in all the samples, except product 6 and 8, out of 10 samples 5 products are accidentally exceeded the permitted values in Food Standards Regulations [1-6].

## Conclusion

The analyses of Benzoic Acid and Sorbic Acid content in 100 samples of different food products were performed by using RP-HPLC. This method enables the simultaneous and selective analyses of these popular preservatives in different types of products. Among them 65% of samples do not compliance with Food Standard Regulation.

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