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Analysis of Sodium Benzoate Levels in Commercial Jellies and Jams using High-Performance Liquid Chromatography

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Abstract. The use of food additives, when posing minimal risk to human health, is generally considered acceptable, as it is a common practice in food production. Among the commonly used food additives are preservatives. Benzoate compounds are frequently employed as preservatives in food products, with sodium benzoate being a prominent example. Sodium benzoate is a synthetic benzoate compound often preferred as a preservative due to its higher solubility when incorporated into food. This study was conducted at Balai BPOM to analyze the sodium benzoate content in commercial jellies and jams. Four samples of jelly and jam were analyzed for sodium benzoate content using High-Performance Liquid Chromatography (HPLC). Two samples (codes 313 and 321) tested positive for sodium benzoate, while the other two (codes 294 and 311) tested negative. The sodium benzoate content in samples 313 and 321 was determined to be 179.10 mg/kg and 165.35 mg/kg, respectively. Based on PerBPOM No. 11 of 2019, both samples were declared safe for consumption.

Keywords : HPLC, jam, jelly, sodium benzoate

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Introduction

Food encompasses all substances derived from natural, biological, and water sources, whether processed or unprocessed, intended for human consumption as food and beverages. This definition includes food additives, food ingredients, and other materials utilized in the preparation, processing, and manufacturing of food and drinks [1]–[3].

The use of food additives, when posing minimal risk to human health, is generally considered acceptable, as it is a common practice in food production. However, according to Law No. 7 of 1996, the excessive use of additives beyond maximum permissible limits or the use of prohibited substances as additives cannot be justified. Such practices can be detrimental and even endanger the health of consumers [4].

Among the commonly used food additives are preservatives. Preservatives extend the shelf life of food products by inhibiting enzymes, disrupting cellular genetic systems, and damaging the cell walls of microorganisms, thereby preventing rapid spoilage.

Benzoate compounds are frequently employed as preservatives in food products, with sodium benzoate being a prominent example. Sodium benzoate is a synthetic benzoate compound often preferred as a preservative due to its higher solubility when incorporated into food [5], [6]. As a salt derivative of benzoic acid, sodium benzoate is widely used in various food and beverage products, including chili sauce, soft drinks, jelly, and jam.

Jellies and jams are semi-solid food products processed from fruits and sugar, typically characterized by a total solids content of at least 65%. Their raw material composition generally consists of 45 parts fruit and 55 parts sugar. Jellies are specifically prepared from fruit juice, whereas jams are made from crushed fruits (pulp). The desirable characteristics of high-quality jellies and jams include transparency, ease of spreadability, and a distinct aroma and taste reminiscent of the original fruit [3], [7].

Jellies made directly from fruit juice offer a nutritional advantage compared to commercial variants that primarily rely on added chemical essences. Conversely, jams are food products where the safety of sodium benzoate preserva-

tive use warrants careful review, as sodium benzoate is commonly incorporated during their production [7]–[9].

High-Performance Liquid Chromatography (HPLC) represents a significant advancement over classical column liquid chromatography. Its technological developments in columns, more sensitive detectors, and high-pressure pumps contribute to HPLC being a rapid and efficient substance separation system [10], [11]. Many studies have developed and validated HPLC methods for the analysis of benzoate in various food matrices. For example, research has focused on determining sodium benzoate levels in soft drinks, sauces, processed fruit products, and canned foods [9], [12], [13]. These studies consistently demonstrate that HPLC is an effective and accurate tool for monitoring benzoate levels in food products. The sodium benzoate content in jams and jellies can also be analyzed using HPLC instrumentation.

According to the Regulation of the Center for Food and Drug Supervisory Agency (BPOM) of the Republic of Indonesia, PerBPOM No. 11 of 2019, the maximum permissible limit for benzoic acid levels in food and soft drinks is 200 mg/kg. In contrast, the Indonesian National Standard (SNI) 01-0222-1995 sets the maximum limit for sodium benzoate at 1 g/kg. The threshold for the permitted use of preservatives is defined as the concentration at which consumers are not adversely affected by their addition [11], [14].

Based on this case study, it is crucial to educate the public regarding the responsible use of preservatives to prevent exceeding predetermined limits during the production of widely circulated food and beverages. The benzoic acid levels determined by HPLC instrumentation were subsequently compared with the maximum limit requirements stipulated in PerBPOM No. 11 of 2019 concerning Benzoic Acid Levels in Food and Soft Drinks.

Experimental

The equipment utilized in this study included a High-Performance Liquid Chromatography (HPLC) system, an analytical balance, various glassware (volumetric flasks, volumetric pipettes, beakers, dropping pipettes, and vials), micropipettes, syringes, spatulas, Whatman No. 45 filter paper, and filter membranes with a pore diameter of 0.45 μm . The materials consisted of four commercial food

samples: jelly packages with codes 313 and 321, and jam packages with codes 294 and 311. Other materials included parafilm and a standard benzoate solution. Reagents used were distilled water, methanol, and a pH 6.8 buffer solution.

Sample preparation involved homogenizing 1 gram of each sample with distilled water in a 50 mL volumetric flask. The mixture was shaken thoroughly and allowed to stand. The resulting precipitate was then filtered using filter paper. Subsequently, the filtrate was further filtered through a filter membrane with a pore diameter of 0.45 μm . Finally, the prepared filtrate solution was injected into the HPLC system, and the results were compared against the benzoate standard solution.

Result and Discussion

Sodium benzoate is a common food and beverage additive primarily used as a preservative. In food and beverages, benzoic acid, which possesses antimicrobial properties, is formed through the conversion (hydrolysis) of sodium benzoate.

To determine the sodium benzoate levels in packaged jellies and jams, a standard benzoic acid solution was initially prepared for the System Suitability Test (SST-UKS). The SST-UKS was performed to confirm that the High-Performance Liquid Chromatography (HPLC) system was capable of accurately analyzing benzoic acid.

The principle of HPLC involves a detector identifying each component as it elutes from the column, with the results recorded as a chromatogram. In a chromatogram, the retention time indicates the number of components, while the peak area signifies the concentration of each component in the mixture. The function of conducting the SST-UKS is to ensure the system's

readiness for analyzing chromatograms from working standards and samples. The results of the benzoic acid intermediate standard solution SST-UKS by HPLC are presented in Figure 1.

Based on Figure 1, the retention time and peak area information for the benzoic acid standard are presented in Table 1. This table shows that benzoic acid exhibits a stable retention time within the range of 7.100 - 7.200 minutes. This consistent retention time serves as a crucial reference for identifying the presence of benzoic acid in samples.

Table 1. SST-UKS HPLC results of benzoic acid intermediate standard solution

No.	Sample Name	Retention Time (minutes)	Area (mAU)
1	UKS5	7.197	336,491
2	UKS6	7.182	337,464
3	UKS7	7.175	337,216
4	UKS8	7.175	336,931
5	UKS9	7.175	338,114

The results from the HPLC analysis of the benzoic acid working standard (WS-BK) solution are depicted in Figure 2. From this figure, details regarding the concentration, retention time, and peak area of benzoic acid are provided in Table 2.

The standard curve for the benzoic acid working solution, derived from the correlation between concentration and peak area, is presented in Figure 3. This curve was generated using Microsoft Excel, yielding a linear equation in the form of $y = bx + a$. The curve was constructed from calculations involving the concentration (in ppm), peak area, and retention time of the working standard stock solution. The resulting coefficient of determination (R^2) for the standard curve was 0.9998, indicating excellent linearity and a strong correlation for benzoic acid, with an equation of $y = 72,015x - 9513.8$.

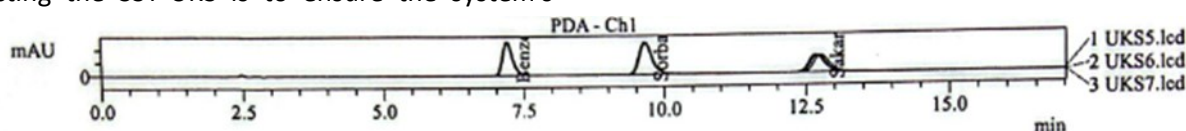


Figure 1. HPLC chromatogram of SST-UKS

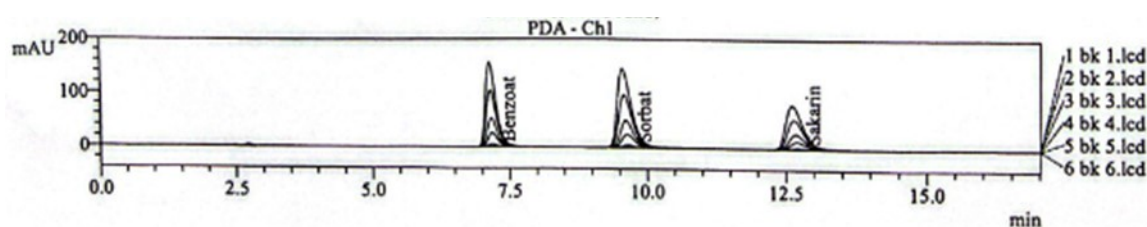
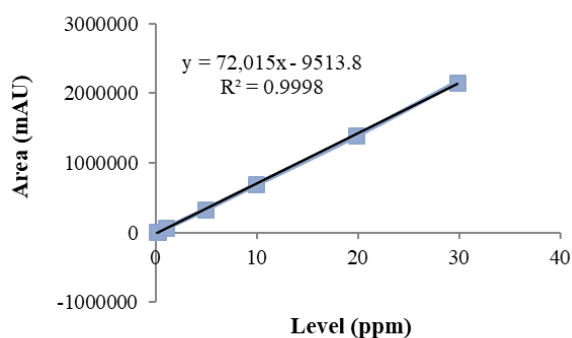


Figure 2. HPLC chromatogram of the WS-BK solution

Table 2. Analysis results of benzoic acid WS-BK solution by HPLC

No	Dilution	Level (ppm)	Area (mAU)	Retention Time (minutes)
1.	1,000	0.0994	8,132	7.189
2.	500	0.1987	13,844	7.184
3.	100	0.9935	67,190	7.184
4.	20	4.9675	337,905	7.182
5.	10	9.9350	695,100	7.166
6.	5	19.8701	1,395,650	7.145
7.	3	29.8051	2,159,158	7.129

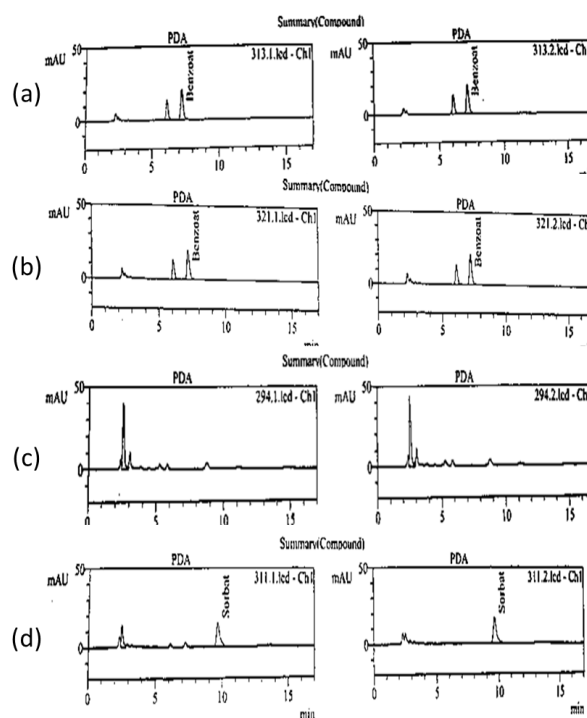
**Figure 3.** Standard curve of benzoic acid WS-BK solution

Following the preparation of the standard curve, test solutions were prepared from the jelly samples (codes 313 and 321) and jam samples (codes 294 and 311). Each test solution was prepared in duplicate to ensure the reproducibility and reliability of the analytical results. The chromatograms from the analysis of these test solutions are presented in Figure 4.

Table 3 provides information on the retention time and peak area obtained from the analysis of the test solutions. A comparison with the standard solution revealed that test solutions with codes 313 and 321 exhibited similar chromatograms and retention times. This similarity strongly indicates the presence of benzoic acid in these samples. Conversely, the test solutions with sample codes 294 and 311 showed no detectable retention time or peak area, confirming that these samples were negative for benzoic acid.

Based on the HPLC analysis, two samples were found to contain detectable levels of sodium benzoate: sample 313 with an average concentration of 179.10 mg/kg, and sample 321 with an average concentration of 165.35 mg/kg. The

detailed results of the benzoic acid level tests using HPLC are presented in Table 4.

**Figure 4.** HPLC chromatograms of: (a) Sample 313, (b) Sample 321, (c) Sample 294, (d) Sample 311**Table 3.** Results of retention time and area by HPLC

No.	Sample Name	Retention Time (min)	Area (mAU)
1.	WS-BK 1	7.189	8,132
2.	WS-BK 2	7.184	13,844
3.	WS-BK 3	7.184	67,190
4.	WS-BK 4	7.182	337,905
5.	WS-BK 5	7.166	695,100
6.	WS-BK 6	7.145	1,395,650
7.	WS-BK 7	7.129	2,159,158
8.	313.1	7.203	260,527
9.	313.2	7.196	260,951
10.	321.1	7.200	253,764
11.	321.2	7.198	268,518
12.	294.1	0.000	0
13.	294.2	0.000	0
14.	311.1	0.000	0
15.	311.2	0.000	0

According to PerBPOM No. 11 of 2019, the maximum permissible limit for benzoic acid levels in food is 200 mg/kg. When the benzoic acid levels in the detected samples are compared against this

Table 4. Test result data of benzoic acid content using HPLC

No. Sample	Weight Sample (g)	Dilution (mL)	Area (mAU)	Retention Time (minutes)	Levels (mg/kg)	Average (mg/kg)
313.1	1.0822	50	260,527	7.200	173.25	179,10
313.2	1.0153	50	260,951	7.198	184.95	
321.1	1.0871	50	253,764	7.200	168.15	165,35
321.2	1.1876	50	268,518	7.198	162.54	
294.1	1.0642	50	-	-	-	-
294.2	1.1455	50	-	-	-	
311.1	1.0223	50	-	-	-	-
311.2	1.1594	50	-	-	-	

PerBPOM regulation, both samples are deemed safe for consumption [11], [14].

Excessive long-term consumption of sodium benzoate can lead to several adverse health effects in the human body. These include the potential development of Systemic Lupus Erythematosus (SLE), edema (swelling) due to fluid retention and increased blood pressure resulting from sodium's osmotic effect on plasma volume. Furthermore, sodium benzoate has been implicated as a carcinogenic agent, potentially increasing the risk of cancer. Other reported negative effects include allergic reactions, neurological diseases, DNA damage, and, in severe cases, it can cause death, with associated symptoms such as hyperactivity, mouth ulcers, constant urination, and significant weight loss [7]–[9], [15], [16].

Conclusion

In conclusion, the High-Performance Liquid Chromatography (HPLC) analysis of four duplicate samples, performed to identify sodium benzoate content, revealed that two samples (codes 321 and 313) tested positive for benzoic acid. Conversely, samples with codes 294 and 311 were determined to be negative for benzoate (preservative). The detected benzoic acid concentrations were 165.35 mg/kg for sample 321 and 179.10 mg/kg for sample 313. Based on PerBPOM No. 11 of 2019, both detected samples are declared safe for consumption.

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Author Contributions

Dedi Wahyu Ari Setiawan: Writing – original draft, visualization, investigation, data curation. M. Mahfudz Fauzi Syamsuri: Writing – review, data curation, editing. Dita Herfiani: Data analysis.

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