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Analysis of Sodium Benzoate Content in Sauces Circulating in Segiri Market Samarinda City by UV-Vis Spectrophotometric Method

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Abstract

Sodium benzoate is one of the permitted preservatives, if the amount is still below the maximum limit. This study was conducted to identify sodium benzoate levels in sauces circulating in Segiri Market, Samarinda. According to BPOM RI regulation No.36 of 2013, the maximum amount of sodium benzoate used in sauce is 1000 mg/kg. In this study, a qualitative analysis of sodium benzoate was carried out using the $FeCl_3$ reagent, where 10 sauce samples taken from the Segiri market in Samarinda City were positive of containing sodium benzoate, forming a salmon-colored or brownish-red precipitate. Quantitative analysis was performed using a UV-Vis spectrophotometer where sodium benzoate was measured at a maximum wavelength of 226 nm. The verification parameters tested in this study are accuracy test and precision test. Based on the verification parameters, the average % recovery was 94.505% which is still in the range of 80-110%, and %RSD of 0.0642% which is less than 2%. Based on quantitative analysis, sample A content was 2,084.8 mg/kg, sample B was 1,895.1 mg/kg, sample C was 2,547.4 mg/kg, sample D was 1,700.7 mg/kg, sample E was 1,466 mg/kg, sample F was 7. 100.1 mg/kg, sample G by 1,388.1 mg/kg, sample H by 1,587.6 mg/kg, sample I by 1,647.1 mg/kg, sample J by 3,172.9 mg/kg, the sodium benzoate content in the sauce samples above the maximum amount as determined by the BPOM regulation No. 36 of 2013.

Keywords: Sodium benzoate, Preservative, Sauce, UV-Vis Spectrophotometry

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1 Introduction

Many food products nowadays contain Food Additives (BTP). According to Permenkes 2012, Food Additives are ingredients added to food to affect the nature or form of food. Food additives, which may or may not have nutritional value, are intentionally added to food for technical purposes during manufacture, processing, handling, packaging, storage or transportation to produce or be expected to produce ingredients or food properties that affect food, either directly or indirectly [1].Preservatives are one of the most commonly used food additives. Preservatives in food aim to make food appear higher quality, more durable and have a more perfect taste and texture [2]. Sodium benzoate is commonly used to preserve various foods and beverages [3]. In food, sodium benzoate breaks down into an effective, undissociated form of benzoic acid [4].

Preservatives can be used if they are in small amounts or within the permitted range. If preservatives are used beyond the permitted limits, they can cause health problems. The growth of the food industry in Indonesia has also led to an increase in the production of food products circulating in the community. Most foods and snacks on the market contain preservatives such as sodium benzoate, borax, formalin, and others [5]. One of the foods that contain preservatives is sauce. Sauce is a paste-shaped product made from raw fruits or vegetables with a pungent aroma and flavor [6]. Sauce is a popular complementary food in the community because it adds flavor to food [7].

The shelf life of a food product depends on good processing techniques and the amount of preservatives used. According to the Regulation of the Head of the Indonesian Food and Drug Administration (BPOM) No. 36 of 2013, the maximum amount of sodium benzoate used in sauces is 1000 mg/kg [8]. The amount of preservatives allowed is the limit at which consumers will not be poisoned by the addition of these preservatives. The addition of preservatives is risky for the health of the body and can cause carcinogenic if it continues to accumulate over time [9].

Based on this background, it is necessary to conduct research to determine the level of sodium benzoate in sauce that is safe in food according to the Regulation of the Head of BPOM RI No. 36 of 2013 is 1000 mg / kg. The selection of sauce as the object of research is because sauce is a common food in the community. Researchers are interested in conducting research on the analysis of sodium benzoate preservative levels in sauces circulating in Segiri Market, Samarinda city. Analysis of sodium benzoate in the sauce was carried out by UV-Vis spectrophotometry.

2 Methods

2.1 Tools and Material

The tools used in this research are UV-Vis Spectrophotometry (Bel Photonics UV-M51®), analytical balance (Fujitsu®), beaker glass (Pyrex®), volume pipette (Iwaki®), separatory funnel (Pyrex®), measuring cup (Pyrex®), volumetric flask (Pyrex®), glass funnel (Pyrex®), dropper pipette, horn spoon, water bath, porcelain cup.

The materials used in this study were sauce samples, $FeCl_3$ 5%, saturated NaCl (Merck®), NaOH 10% (Merck®), HCl 0.1% (Merck®), 70% ethanol, 96% ethanol p.a, blue litmus paper, filter paper, sodium benzoate p.a, chloroform, diethyl ether, concentrated HCl, hot water, aquadest, aluminum foil.

2.2 Qualitative Analysis

Each sauce weighed as much as 20 g of sample and was put into a glass beaker plus saturated NaCl to a volume of 100 mL, then the sample was added with 10% NaOH solution until the solution was alkaline, stirred for 5 minutes, left for 2 hours, and filtered. The filtrate was added with 2 mL of concentrated HCl solution until the solution was acidic. The

acid solution was extracted with chloroform 3 times for 10 mL each, then the chloroform extract was heated in a water bath at 80°C. The residue obtained was dissolved in *distilled* water and heated in a water bath for 10 minutes at a temperature between 80-85°C. The solution obtained was cooled for a moment and a few drops of 5% $FeCl_3$ solution were added. If a salmon-colored precipitate or brownish-red ring is formed, it indicates the presence of benzoate in the sample [10].

2.3 Quantitative Analysis

2 g samples were weighed carefully and put into a 100 mL beaker then added saturated NaCl solution up to 20 mL, added with HCl until acidic (blue litmus paper becomes red) then homogenized, then the solution was put into a separating funnel, first extracted with 7.5 mL diethyl ether until two layers were formed. Next, the lower layer or water layer is separated then the upper layer or ether layer is washed with 0.1% HCl, 3 times with 5, 4, and 3 mL respectively. The lower layer was discarded and the upper layer was washed. The ether extract was put into a 50 mL volumetric flask and added to the limit with 70% ethanol, then diluted with 96% ethanol in a 100 mL flask. The solution obtained was read for absorbance using UV-Vis spectrophotometry at the maximum wavelength [11].

2.4 Preparation of 100 ppm sodium benzoate stock solution

10 mg of sodium benzoate is added into 100 mL volumetric flask then dissolved with 96% ethanol.

2.5 Determination of sodium benzoate maximum wavelength

Sodium benzoate standard solution of 10 mg/mL was taken 6 mL and put into a 10 mL volumetric flask, then dissolved with 96% ethanol so that a solution with a concentration of 60 ppm was obtained. Then, the absorbance of the solution was measured with a UV-Vis spectrophotometer at a wavelength of 200-400 nm. As a result of the measurement, the maximum wavelength of the sodium benzoate standard was obtained.

2.6 Calibration solutions preparation

From the sodium benzoate stock solution following dilutions were prepared: 15, 30, 45, 60, and 75 ppm. Put into each 10 mL volumetric flask 1.5, 3, 4.5, 6, and 7.5 mL of stock solution. Then diluted with 96% ethanol to obtain concentrations of 15, 30, 45, 60, and 75 ppm. Each standard solution was measured for absorbance at a wavelength of 226 nm [12].

2.7 Determination of sodium benzoate content in sauce

The sodium benzoate content in the sauce sample can be calculated using the following formula in Equation 1.

$$\text{Level} = \frac{C \times V \times fp}{W} \quad \text{(Equation 1)}$$

Description:
C = Concentration
V = Total sample volume (L)
Fp = Dilution Factor
W = Sample Weight (kg)

2.8 Method Validation

2.8.1 Precision Test

The precision test was carried out by measuring the absorbance of each sample at the maximum wavelength (226 nm) using UV-Vis Spectrophotometer . The sample measurements were repeated 3 times. Then the sample concentration was measured using the linear regression equation y = 0.0348x + 0.2128 and the RSD value was calculated. As required, the precision measurement yields acceptable results, with a %RSD value of less than 2% [13].

2.8.2 Accuracy Test

The accuracy test was carried out using the standard addition method. The method is done by taking a sample solution of 5 mL and adding a standard solution with different concentrations for each sample. Sample A with a concentration of 10.424 ppm was 0.521 mL, Sample B with a concentration of 9.475 ppm was 0.473 mL, Sample C with a concentration of 12.737 ppm was 0.636 mL, Sample D with a concentration of 8.503 ppm was 0.425 mL, Sample E with a concentration of 7.33 ppm was

0.366 mL, Sample F with a concentration of 35.500 ppm as much as 1.775 mL, Sample G with a concentration of 6.940 ppm as much as 0.347 mL, Sample H with a concentration of 7.938 ppm as much as 0.396 mL, Sample I with a concentration of 8.235 ppm as much as 0.411 mL, Sample J with a concentration of 15.864 ppm as much as 0.793 mL. Then homogenized and measured the absorbance at a wavelength of 226 nm, then calculate the % *recovery* value. The accuracy test is acceptable if the % recovery obtained is in the range of 80-120% [13].

3 Results and Discussions

3.1 Qualitative Test

This qualitative analysis is carried out to determine the presence or absence of sodium benzoate content in sauce samples, sauce samples that are declared to contain sodium benzoate form a salmon or brownish red precipitate (Table 1) [10].

Table 1. Qualitative analysis of sodium benzoate in samples

No	Sample	Identification Results ($FeCl_3$ 5%)	Results
1	Positive control	Salmon- colored or brownish-red sediment	+
2	A	Salmon- colored or brownish-red sediment	+
3	B	Salmon- colored or brownish-red sediment	+
4	C	Salmon- colored or brownish-red sediment	+
5	D	Salmon- colored or brownish-red sediment	+
6	E	Salmon- colored or brownish-red sediment	+
7	F	Salmon- colored or brownish-red sediment	+
8	G	Salmon- colored or brownish-red sediment	+
9	H	Salmon- colored or brownish-red sediment	+
10	I	Salmon- colored or brownish-red sediment	+
11	J	Salmon- colored or brownish-red sediment	+

Qualitative analysis was carried out with reference to the method of [10], by using the $FeCl_3$ reagent. In the qualitative analysis of the sample, the purpose of adding saturated NaCl is to break the emulsion of the sauce, because the addition of electrolytes will break the emulsion [14]. In the next step, stirring is needed to make the solution homogeneous. After that, the solution was left to stand for 2 hours, water-insoluble dispersed particles such as fat will precipitate in the form of fatty acid salts. Filtering is done to separate the water-insoluble particles from the solution. Benzoate will exist as a salt in the aqueous solution in the filtrate [10]. Then HCl is added until it is acidic to convert the sodium benzoate salt into benzoic acid, then extracted with chloroform.

After extraction, two separate layers are divided. The top layer is the water phase, while the bottom is the chloroform phase. This is because the specific gravity of chloroform (ρ = 1.479 g/ml) is higher than the specific gravity of water (ρ = 1 g/ml). The solvent was then evaporated on a water bath at 80°C, leaving a residue. The resulting residue was dissolved in distilled water and heated in a water bath at 80-85°C for 10 minutes. The solution was then allowed to cool for a while and a few drops of 5% $FeCl_3$ were added. The addition of $FeCl_3$ 5% will form a salmon-colored or brownish-red precipitate. The formation of a salmon-colored or brownish-red precipitate on the residue indicates that the positive (+) sample contains sodium benzoate. The precipitate formed is iron (III) benzoate or ferric benzoate [14], [15].

3.2 Quantitative Test

3.2.1 Wavelength Determination

Determination of the maximum wavelength aims to find out at what wavelength produces the highest absorbance value. This is because if measurements are taken at the same wavelength, the data obtained will be more accurate. Determination of wavelength is done by measuring the concentration of 60 ppm solution in the wavelength range of 200-400 nm. The maximum wavelength of sodium benzoate measured was 226 nm.

3.2.2 Calibration Curve

The standard curve is the ratio between concentration and absorbance value. The greater the concentration, the greater the absorbance. In this study, the sodium benzoate standard curve was made with several variations in sodium benzoate standard concentration. The concentrations required were 15, 30, 45, 60, and 75 ppm with 96% ethanol solvent, then measured at a wavelength of 226 nm.

Making a standard curve aims to determine the relationship between the concentration of the solution and its absorbance so that it can determine the concentration of a substance in an unknown sample. The results of the standard absorbance of sodium benzoate at each concentration can be seen in Table 2. From the results of the table shows that between the

concentration and absorbance where the higher the concentration, the higher the absorbance value produced. From the sodium benzoate standard curve, the absorbance of the solution concentration is obtained which is the relationship between the x-axis and the y-axis. The x-axis is the concentration and the y-axis is the absorbance obtained from the measurement results so that the linear regression equation of the calibration curve is y = 0.0348x + 0.2128 with a correlation coefficient (r) = 0.9973 (Figure 1).

Table 2. Absorbance value of sodium benzoate standard solution

Concentration (ppm)	Absorbance
15	0,682
30	1,313
45	1,811
60	2,287
75	2,807

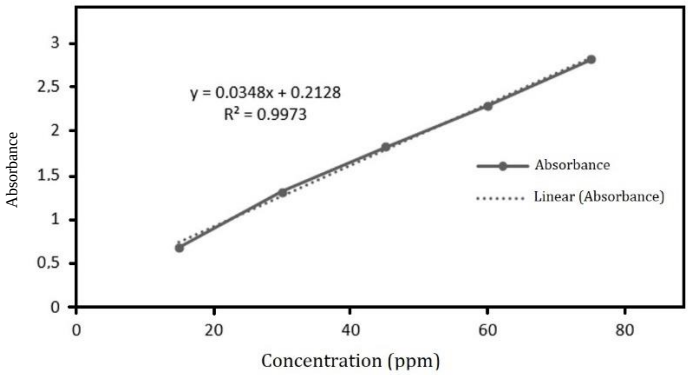

Figure 1. Calibration curve of sodium benzoate in ethanol at 226 nm wavelength

3.2.3 Determination of Sodium Benzoate Level

The quantitative test aims to determine the level of sodium benzoate in the sample, by measuring the absorbance of the sample using UV-Vis spectrophotometry at a wavelength of 226 nm, then calculating the level.

Based on the results of this study contained in table 3 shows that the concentration of sodium benzoate in sample A was 2,084.8 mg/kg; sample B was 1,895.1 mg/kg; sample C was 2,547.4; sample D was 1,700.7; sample E was 1,466; sample F was 7,100.1; sample G was 1,388.1; sample H was 1,587.6; sample I was 1,647.1; sample J was 3,172.9. Based on the analysis of the 10 samples obtained, it shows that the sodium benzoate level exceeds the threshold set in BPOM RI regulation No. 36 of 2013, which is 1000 mg/kg [8].

Table 3. Sodium benzoate levels in sauce samples

Sample	Level (mg/kg)
A	2.084,8
B	1.895,1
C	2.547,4
D	1.700,7
E	1.466
F	7.100,1
G	1.388,1
H	1.587,6
I	1.647,1
J	3.172,9

3.3 Verification Method

3.3.1 Precision

The test was carried out by measuring the absorbance of the sample that had been prepared and each sample was replicated three times, then the sample concentration was calculated using the linear regression equation y = 0.0348x + 0.2128. The test results of standard deviation (SD) and relative standard deviation (RSD) can be seen in table 4. Based on the results in table 4, the relative standard deviation (RSD) value is 0.0642%. RSD in this test is still included in the requirements because based on the literature the coefficient of variation is less than 2% [13].

Table 4. Precision test results

Sampel	Concentration (ppm)	SD	RSD (%)
A	20,848	0,0076	0,036
B	18,951	0,0079	0,041
C	25,474	0,0079	0,031
D	17,007	0,0078	0,045
E	14,660	0,0134	0,091
F	71,001	0,0313	0,044
G	13,881	0,0209	0,150
H	15,876	0,0079	0,049
I	16,471	0,0136	0,082
J	31,729	0,0234	0,073
Average		0,01417	0,0642

3.3.2 Accuracy

Accuracy is a validation parameter that shows the similarity obtained from the analysis results with the actual analyte. Accuracy is expressed as a percent recovery of the added analyte [13]. In this study using the standard addition method. The results obtained are the *spiking* absorbance which can be seen in Table 5. Then calculated using the linear regression equation y = 0.0348x + 0.2128 and obtained the *spiking* concentration to calculate the % *recovery*.

Table 5. Spiking absorbance results

Sample	Absorbance sample	Standard solution addition (mL)	Absorbance spiking
A	0,938	0,521	1,286
B	0,872	0,473	1,186
C	0,099	0,636	1,515
D	0,805	0,425	1,083
E	0,723	0,366	0,963
F	2,683	1,775	3,825
G	0,695	0,347	0,927
H	0,765	0,396	1,027
I	0,786	0,411	1,055
J	1,317	0,793	1,844

Table 6. Recovery of sodium benzoat to evaluate the accuracy of the UV method

Sampel	Spiking concentration (ppm)	*%recovery*
A	30,839	95,846%
B	27,965	95,134%
C	37,419	93,781%
D	25,005	94,060%
E	21,557	94,092%
F	103,798	92,385%
G	20,522	95,691%
H	23,396	94,734%
I	24,201	93,867%
J	46,873	95,461%
Average	36,157	94,505%

Table 5 shows that the absorbance value of spiking is higher than the absorbance of the sample. Spiking is the addition of standard solution to the sample solution. The results of the calculation can be seen from table 6 showing the average *recovery* (% recovery) of sodium benzoate, namely, 94.505%. The *recovery* (% *recovery*) shows good accuracy of sodium benzoate levels in the sample. The mean sodium benzoat recovery were close to 100%. The % *recovery* test results are acceptable because they meet the predetermined accuracy requirements, namely, in the range of 80-110% [13].

4 Conclusions

Qualitative analysis of sodium benzoate was carried out using the $FeCl_3$ reagent, where 10 sauce samples taken from the Segiri market in Samarinda City were positive of containing sodium benzoate, forming a salmon-colored or brownish-red precipitate. Quantitative analysis was performed using a UV-Vis spectrophotometer where sodium benzoate was measured at a maximum wavelength of 226 nm. Based on the results of quantitative analysis showed that 10 samples of sauce circulating in Segiri Market, Samarinda City above the standard limit determined by BPOM RI regulation No. 36 of 2013 which is 1000 mg/kg. Sodium benzoate levels in sample A were 2,084.8 mg/kg, sample B was 1,895.1 mg/kg, sample C was 2,547.4 mg/kg, sample D was 1,700.7 mg/kg, sample E was 1,676.4 mg/kg, and sample F was 1,676 mg/kg, sample G was 1,388.1 mg/kg, sample H was 1,587.6 mg/kg, sample I was 1,647.1 mg/kg, sample J was 3,172.9 mg/kg. Based on the verification parameters, the average % recovery was 94.505% which is still in the range of 80-110%, and %RSD of 0.0642% which is less than 2%.

5 Declarations

5.1 Funding

This research was not supported by any funding sources.

5.2 Authors Contributions

The names of the authors listed in this journal contributed to this research.

5.3 Conflict of Interest

The authors declare no conflict of interest.

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