

The Analysis of Benzoate in Deepika Sauce of Sejahtera Sentosa SME with Uv-Vis Spectrophotometry Method

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ABSTRACT

The utilization of food additives, particularly benzoate as preservatives, must adhere to Indonesian regulations to guarantee the safety of food for consumers. This study aims to detect the presence of benzoate compounds and quantify their concentrations in six varieties of sauces manufactured by SME Sejahtera Sentosa. Benzoate compounds are identified by forming a brownish precipitate with FeCl₃, while their concentrations are measured using UV-Vis spectroscopy at a wavelength of 274 nm. Results reveal that all six samples of Deepika sauces A, B, C, D, E, and F contain benzoate preservatives at concentrations of 0.986, 0.943, 0.852, 0.635, 0.700, and 0.992 g/kg, respectively. The levels of benzoate preservatives in the sauce samples remain within the limit established by Regulation Number 11 of 2019 by the Head of the Indonesian Food and Drug Monitoring Agency, which is 1 g/kg.

Keywords: benzoate preservatives, sauces, ultraviolet-visible spectrophotometry

INTRODUCTION

A sauce is a paste-based product made from fruit or vegetable-based raw materials which has an appetizing aroma and taste (Yazdanfar, Manafi, Ebrahiminejad, et al., 2023). Sauces commonly sold in Indonesia are tomato sauce and chili sauce (Rahmania et al., 2020). There are also various types of sauces manufactured, such as those manufactured by Sejahtera Sentosa SME, which obtained a halal license in 2022 and has produced 6 types of sauces, namely Deepika Cheese Sauce, Deepika Lemon Sauce, Deepika Sweet Chili Korean Sauce, Deepika Jalapeno Sauce, Deepika BBQ Sauce, Deepika Black pepper Sauce. This variety of sauce has obtained a positive reaction from the public, which is in line with the rapid production of sauces due to the increase in people's demand, according to the Center of Agricultural Data and Information, which reports that the average consumption of tomato sauce or chili sauce is 140 ml/week (Aripriharta et al., 2021).

The sauce taste typically varies depending on the spices added, while the red color of tomato sauce corresponds to the red color of the raw material (Yuwindry, 2020). Tomato sauce is a semi-solid product that is widely consumed as a complement to dishes, in the form of a thick paste attained from the processing of tomatoes mixed with sugar, acid, vinegar, salt, thickening agents, and other ingredients such as coloring agents and preservatives (Jannah et al., 2021; Yazdanfar, Manafi, Ebrahiminejad, et al., 2023).

The growing production of Deepika sauce must be in line with the safety of the sauce product, which must also receive attention, both from the aspect of sanitary hygiene and the food additives added. Food additives are ingredients added to food to change the nature or form of food. The use of this material is not for direct consumption. Preservatives are food additives to prevent or inhibit fermentation, acidification, or other decomposition of food caused by microorganisms (Pongsetkul & Benjakul, 2021). Preservatives are usually added to preserve perishable food ingredients with the function of maintaining food nutrition and increasing food shelf life (Sukumaran & Radhakrishnan, 2021). Preservatives that are widely used in the market are benzoates, such as sodium benzoate, since their solubility is better than other forms (Ravianda et al., 2022).

Sodium benzoate ($C_7H_5O_2Na$) is a sodium salt that inhibits the growth of microorganisms (Chiple, 2020). Sodium benzoate is more widely used in food and beverage products due to its stability and good solubility in water. Exposure to sodium benzoate is generally through food. Once enters the digestive tract, sodium benzoate will be rapidly absorbed. After absorption through the digestive tract or skin, sodium benzoate will be metabolized in the liver through conjugation with glycine and later produce hippuric acid. Hippuric acid is then excreted in the urine (Chiple, 2020; Khalili & Khaniki, 2023; Sabour & Ibrahim, 2019; Yazdanfar, Manafi, Rafsanjani, et al., 2023). In humans, consuming sodium benzoate at a dose of up to 160 mg/kg, 75-100% of the dose will be excreted within 6 hours, while the rest will persist for 2-3 days before being excreted (Islam et al., 2022)

Furthermore, sodium benzoate consumption can affect body functions and metabolic processes involving glycine. Urticaria, asthma, rhinitis, or anaphylactic shock have been reported in people with a history of atopy (Khan et al., 2020). Research by Jannah et al. (2021) stated that two out of three sauce samples in Malang City tested had benzoate preservative levels exceeding the maximum limit set of 1 gram per kilogram. Findings from other studies also indicate that all samples, or 100% of all tested samples, had benzoate content exceeding the established maximum limit (Rianto et al., 2020).

The safe limit for adding sodium benzoate to food is regulated by various organizations worldwide. According to regulations in Indonesia, the maximum limit of sodium benzoate content in food is 1 g/kg (Esrafil et al., 2022; Ravianda et al., 2022). Employing the UV-Vis Spectrophotometry method, this research aims to provide a deeper understanding of the

benzoate content in 6 types of Deepika SME Sejahtera Sentosa sauces, including Jalapeno, Korean Sweet Chilli, Black Pepper, Lemon, Barbeque, and Cheese, which have received positive feedback from the general public. Additionally, this research is dedicated to consumer safety and contributes to developing more accurate and efficient analytical methods for monitoring food safety.

MATERIAL AND METHOD

Material

The tools employed were Uv-Vis spektrofotometer, analytical balance (Ohaus pioneer), separator funnel (pyrex), measuring flask (pyrex), Erlenmeyer (pyrex) and glassware. The materials used are of proanalysis quality, namely 6 types of Deepika sauces from SME Sejahtera Sentosa, sodium benzoate (Onemed), chloroform (Emsure), sodium hydroxide (Merck), iron (III) chloride (Merck), hydrochloric acid (Emsure), sodium chloride (Merck), ammonium hydroxide (Emsure), sodium sulfate (Onemed), distilled water, litmus paper, and filter paper (Onemed).

Qualitative Analysis

Qualitative analysis method was in accordance with the modified (Jannah *et al.*, 2021). A total of 20 g of sample was put into a glass beaker to a volume of 100 mL with saturated NaCl solution. Then the sample was added with 10% NaOH solution until the solution was alkaline, stirred for 5 minutes, left overnight and filtered. The filtrate was added with 2 mL of concentrated HCl solution until the solution was acidic. The acid solution was extracted 3 times with 10 mL of chloroform each, then the chloroform extract was heated at 80°C over a water bath. The residue obtained was dissolved in distilled water and heated in a water bath for 10 minutes between 80-85°C. Then, the solution obtained was briefly cooled and added a few drops of 5% FeCl₃ solution. If a salmon-colored precipitate or a red-brown ring is formed, this indicates the presence of benzoate in the sample.

Standard Curve

The standard solution preparation was preceded by the preparation of a 100 mg/L mother liquor by dissolving 25 mg of sodium benzoate in chloroform in a 250 mL measuring flask. Standard series solution was prepared by taking: 1; 2; 3; 4; and 5 mL of 100 mg/L sodium benzoate mother liquor into a 10 mL measuring flask and then each diluted with chloroform up to the mark. The concentrations of the standard solutions obtained successively were: 10; 20; 30; 40; and 50 mg/L.

The absorbance detection of the standard solution was carried out at a wavelength of 274 nm using a UV-Vis spectrophotometer. Then a standard curve was prepared that related the absorbance to the concentration of each standard solution (Rohmah *et al.*, 2021).

Precision

From the standard sodium benzoate solution of 100 mg/L, a standard solution with a concentration of 40 mg/L was prepared in the same way as the concentration series on the standard curve. The standard sodium benzoate solution with a concentration of 40 mg/L was read for its absorbance at a wavelength of 274 nm. This accuracy test was carried out five times (Pravalika & Archana, 2022).

Accuracy

The accuracy was determined using standard addition method to a sodium benzoate solution of known concentration, which was added to a standard sodium benzoate solution of 5 mg/L.

Then the absorbance of the solution was read using a UV-Vis spectrophotometer with 5 repetitions. The absorbance results are employed to calculate the percent recovery (Pravalika & Archana, 2022).

LOD and LOQ

LOD and LOQ were calculated through the linear equation of the calibration curve with the formula:

$$LOD=(3 \times SB)/slope \tag{1}$$

$$LOQ=(10 \times SB)/Slope \tag{2}$$

Note:

SB = Standard deviation of the analytical response from the blank

Slope = The linear line direction (sensitivity direction) of the curve between the response to concentration = slope (b in the equation of the line $y = bx + a$) (Pravalika & Archana, 2022).

Quantitative Analysis

Quantitative analysis method was in accordance with AOAC (Horwitz, 2005) and modified by Jannah *et al.*, (2021). A total of 10 grams of sample was transferred into an Erlenmeyer, then the sample was dissolved in 100 mL of saturated NaCl solution. A few drops of HCl were added until the solution was acidic (blue litmus paper turned red) then mixed well. The solution was extracted with chloroform 3 times each: 30, 20, 10 mL. The extraction results were washed with 0.1% HCl solution 3 times, each 25, 20, and 15 mL. The acid extract was extracted again with 0.1% NH4OH solution 4 times each: 25, 20, 15, 10 mL. Then, the extraction results were extracted with chloroform 3 times, respectively: 30, 20, and 10 mL. The extraction results were washed with Na2SO4 and diluted with chloroform up to the mark in a 100 mL measuring flask.

The absorbance of the extracted solution was read using a UV-Vis spectrophotometer at a wavelength of 274 nm, then the concentration of benzoate in the sample was determined based on the standard curve.

RESULT AND DISCUSSION

The qualitative analysis carried out on the sample aims to illustrate the presence of benzoate compounds in the sample. The results of a qualitative analysis of the presence of benzoate in some chili sauce samples can be viewed in Table 1.

Table 1. The qualitative analysis results of benzoic acid presence in Deepika sauce samples

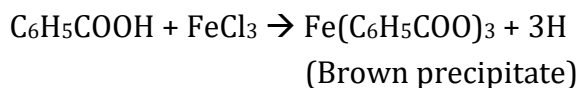
Sample	Result	Explanation
Jalapeno	+	Brown precipitate
Korean Sweet Chilli	+	Brown precipitate
Blacpepper	+	Brown precipitate
Lemon	+	Brown precipitate
Barbeque	+	Brown precipitate
Keju	+	Brown precipitate

Based on Table 1, all samples showed positive presence of benzoate compounds. Qualitative analysis results, indicated by the formation of salmon-colored precipitate or the presence of reddish-brown rings (Chiple, 2020).

In a sample of 20 g of sauce, mixed with a saturated NaCl solution with the aim of breaking the emulsion of chili sauce. The addition of electrolyte aims to facilitate emulsion breaking. Further stirring is done to achieve solution homogeneity. After allowing the solution to stand overnight, dispersed particles insoluble in water, such as fats, will precipitate in the

form of fatty acid salts. Filtration process is carried out to separate insoluble particles from the solution. Benzoate will dissolve in the aqueous solution in the filtrate form of salt (Pramitha *et al.*, 2020).

The mixture of chloroform extract tested with the addition of 5% FeCl₃ reagent produces a reddish-brown precipitate or salmon-colored ring. This is caused by the formation of bonds between three benzoate ions from benzoic acid and ferric ions (Fe³⁺) from ferric chloride, forming ferric benzoate chelate compounds with hydrochloric acid molecules (Azmi *et al.*, 2020; Pramitha *et al.*, 2020), according to the reaction.i :



Scheme 1. Ferric chloride chelate reaction

Kurva Standar dan Linieritas

In this study, the standard curve was obtained by creating a series of concentrations of 10, 20, 30, 40, and 50 mg/L from a standard solution of 100 mg/L sodium benzoate so that the absorbance towards concentration results were obtained at a wavelength of 274 nm.

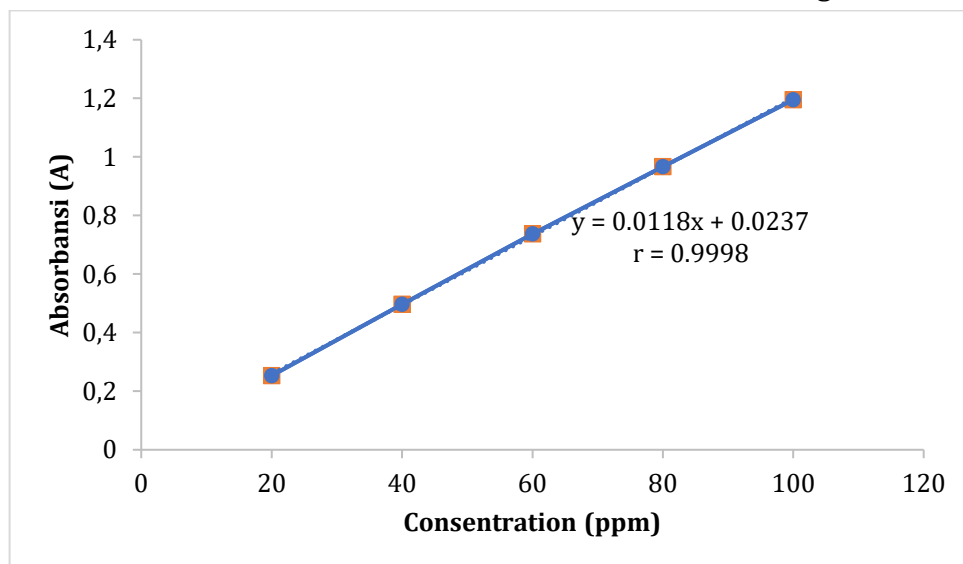


Figure 1. Standard curve of sodium benzoate

The calibration curve equation is the correlation between the x-axis and the y-axis. The x-axis is expressed by the concentration obtained while the y-axis is the absorbance obtained from the measurement results so that the linear regression equation of the calibration curve obtained was $y = 0.0118x - 0.0237$ with a correlation coefficient of $r = 0.9998$. The value of the correlation coefficient (r) which was close to 1 indicated a linear correlation between the concentration and the absorption produced (Elmanfe, 2020), in other words the increase in the absorbance value of the analyte was directly proportional to the increase in its concentration which was in accordance with the acceptance criteria for a good correlation coefficient (r), namely $r \geq 0.9970$ (Astuti *et al.*, 2019).

Precision

Precision is measured as standard deviation or relative standard deviation (coefficient of variation) based on research conducted on replicated samples taken from homogeneous mixtures (E. & B., 2021). The method can produce an average value that is very close to the true value with the standard deviation (SD) and the coefficient of variation (KV) as measuring parameters. The results of the calculation of the standard deviation (SD) from the data obtained and 5 times the replication was 0.0026 with a coefficient of variation (KV) of 0.19%. The coefficient of variation <2% indicated that the method provided good precision, so that

the accuracy of the tool obtained was 99.99% (Rohmah *et al.*, 2021). Experimental data results are illustrated in Table 2.

Table 2. Precision Test Results Data on Standard Solutions

Replication	Absorbance	Concentration
1	0.495	39.94
2	0.496	40.03
3	0.497	40.11
4	0.495	39.94
5	0.495	39.94
Average	0.4956	39.99
	SD	0.08

Accuracy

Accuracy can be determined in 2 ways, namely, the simulation method (spiked - placebo recovery) or the standard addition method as employed in this study (Sen *et al.*, 2021). In the standard addition method, with 5 repetitions, the absorbance was read before and after the addition of 5 mg/L sodium benzoate standard solution concentration. After calculation, the average value of % recovery obtained was 84.82%. This recovery percentage was acceptable since it met the accuracy requirements, namely in the range of an average recovery percentage of 80 – 110%.

Tabel 3. Accuracy Test Results Data on Standard Solutions

Data	Concentration before	Concentration after + Standard Solution	Concentration before	Concentration after + Standard Solution	Recovery
1	0.495	0.271	39.94	20.96	84.37
2	0.496	0.271	40.03	20.96	84.75
3	0.497	0.27	40.11	20.87	85.50
4	0.495	0.27	39.94	20.87	84.75
5	0.495	0.27	39.94	20.87	84.75
	Average				84.82

LOD and LOQ

Limit of detection and limit of quantification tests were carried out to determine the lowest limit of detection and quantification of the solution (sodium benzoate) which can still produce data with good accuracy and precision (Pravalika & Archana, 2022). After obtaining a calibration curve that met the requirements of the analysis, then the data obtained from the concentration of each analyte with a different absorbance was processed to determine the limit of detection (LOD) and limit of quantification (LOQ). The detection limit obtained was 0.180 mg/L while the quantification limit obtained was 0.599 mg/L.

Analisis kuantitatif

Determination of benzoate compounds level in the sample was carried out by measuring the absorbance of the sample solution. The concentration (X) of benzoate compounds in the sample was calculated by substituting the absorbance value of the sample solution into the equation $y = 0.0118x + 0.0237$.

Table 4. Measurement results of benzoate compound concentrations in Deepika sauce sample extracts.

Sample	Repetition	Average	Result			
			mg/L	mg/100mL	mg/kg	g/kg
Jalapeno	A1	107.529				
	A2	94.151	98.610	9.861	986.103	0.986±0.07
	A3	94.151				
Korean Sweet Chilli	B1	93.710				
	B2	93.710	94.275	9.428	942.749	0.943±0.01
	B3	95.405				
Blackpaper	C1	85.193				
	C2	85.193	85.193	8.519	851.929	0.852±0
	C3	85.193				
Lemon	D1	64.105				
	D2	62.431	63.547	6.355	635.470	0.635±0.01
	D3	64.105				
Barbeque	E1	70.608				
	2	68.938	70.051	7.005	700.512	0.700±0.01
	E3	70.608				
Keju	F1	99.798				
	F2	99.798	99.237	9.924	992.369	0.992±0.01
	F3	98.115				

The determination of concentrations present in the chili sauce samples can be seen from the average concentration of the samples, which is the sum of the benzoate compound concentrations contained in 10 grams of chili sauce material obtained based on absorbance readings from 100 mL of the final extract in the series of quantitative analysis procedures. The concentration of benzoate compounds in 10 grams of material from 100 mL is converted into units of mg/kg, where 1 kilogram of material is equal to 10 grams of material multiplied by 100.

The variation in benzoate levels in each sauce sample is due to the semi-automatic production of Deepika sauce (Aripriharta *et al.*, 2021). The concentration of benzoate compounds in the 6 samples of Jalapeno, Korean Sweet Chilli, Black Pepper, Lemon, Barbeque, and Cheese sauces based on Table 4, respectively, are 0.986; 0.943; 0.852; 0.635; 0.700; and 0.992 g/kg, indicating that the benzoate compound levels do not exceed the threshold according to the Regulation of the Head of the Indonesian Food and Drug Monitoring Agency Number 11 of 2019 for chili sauce, which is 1g/kg (Manoppo *et al.*, 2019). This research can provide information to the public that Deepika SME Sejahtera sauce is not harmful to public health and is safe for consumption.

CONCLUSIONS

Deepika SME Sejahtera Sentosa sauce contains benzoate compounds. The benzoate levels in Deepika SME Sejahtera Sentosa sauce are as follows: Jalapeno: 0.986 g/kg; Korean Sweet Chilli: 0.943 g/kg; Black Pepper: 0.852 g/kg; Lemon: 0.635 g/kg; Barbeque: 0.700 g/kg; Cheese: 0.992 g/kg. These levels do not exceed the threshold for the use of benzoate compounds as determined in Regulation of the Head of the Indonesian Food and Drug Monitoring Agency Number 11 of 2019.

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