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Determination Of Sodium Nitrite in Dumplings Available in Online Shops Using Ultraviolet-Visible Spectrophotometry

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Abstract

This research aims to determine the sodium nitrite content in dumplings in online stores based on differences in storage. The method used in this research is UV-Vis spectrophotometry with a maximum wavelength of 540 nm. The samples obtained from the online shop were two with different brands and were given the codes MT and CD. Samples in closed packaging that are still sealed are categorised as fresh samples and then tested for sodium nitrite content in the sample. Then, the remaining fresh samples were tested after being stored for 24 hours. The research results showed that the sodium nitrite content in fresh samples of different brands had different levels. Respectively, sodium nitrite levels with codes MT and CD were obtained at 9,890 mg/Kg and 15,545 mg/Kg. Samples that had undergone a 24-hour storage process with the MT and CD brands obtained 6,862 mg/Kg sodium nitrite contents and 13,209 mg/Kg. The sodium nitrite content in samples from different brands is still below the requirements set by the National Agency of Drug and Food Control of the Republic of Indonesia Regulation No. 11 of 2019 concerning food additives, namely 30 mg/Kg. This means that the sample is still categorised as suitable for consumption by the public. Fresh sample precision as %RPD for MT and CD brands is 7.030% and 6.954%. Meanwhile, sample precision after storage for the MT and CD brands was 8.382% and 1.921%, respectively. Thus, the precision in this test is declared to meet the acceptance requirements of <10%. The estimated uncertainty of the test for determining sodium nitrite levels in dumpling samples meets the acceptance requirements of <30% levels.

Keywords*: dumpling, sodium nitrite, spectrophotometry, precision, uncertainty*

Introduction

Nowadays, fast food is a practical alternative that looks attractive and tastes delicious, like frozen food sold in minimarkets, supermarkets, distributor agents, and online shops. Frozen food is a type of food that is frozen to preserve the food until it is consumed. This freezing aims to slow down decomposition by converting the remaining water content into ice to inhibit the growth of most bacterial species (Nessianti & Soeyono, 2015). There are many types of frozen food. One type is dumplings. Dumplings are a typical Chinese food made from processed beef or chicken as filling. A dumpling is a type of dim sum with a cone shape as its characteristic shape (Hardiman, 2017).

The existence of frozen food can be beneficial and can also be detrimental for consumers because it can endanger health. Frozen food is helpful in everyday life because it does not take long to process. However, frozen food can harm consumers because it contains other additives such as preservatives, sweeteners, and colourings. The preservatives used in frozen food are natural or synthetic preservatives such as sodium nitrite. Sodium Nitrite is used to obtain good colour and prevent the growth of microbes such as Clostridium botulinum. Sodium nitrite is useful in meat processing as a colour maker and preservative and as a stimulating factor for aroma and taste. Therefore, the use of this material is increasingly widespread. The use of sodium nitrite as a preservative is permitted; however, the rules for its use in food must not exceed limits and have no impact on human health.

According to the National Agency of Drug and Food Control of the Republic of Indonesia Regulation No. 11 of 2019 concerning food additives, the maximum use of nitrite preservatives in processed meat products and processed meat products is 30 mg/kg (Peraturan Badan Pengawas Obat Dan Makanan Tentang Bahan Tambahan Pangan Nomor 11 Tahun 2019 Tentang Bahan Tambahan Pangan, 2019). If it is excessive, nitrite will react with secondary or tertiary amino acids to form N-nitrosamine compounds, mutagens and carcinogens, and then nitrosamines show carcinogenic activity (Pulung, 2019). Sodium nitrite can form nitrosamine derivatives, which are toxic when combined with amines or amides. Moreover, if sodium nitrite enters the body through food, it can cause Methemoglobinemia, where the blood cannot bind oxygen if the nitrite reacts with hemoglobin, which can result in shortness of breath or lack of oxygen in the body, high doses of nitrite can also produce pseudo-cyanosis, tissue hypoxia, and death (Katzung, 2004).

The method often used to determine sodium nitrite levels in a sample is spectrophotometry. The spectrophotometric method has many advantages compared to other methods such as fluorometry, electrochemistry, polarography, amperometry, chromatography, and potentiometry because spectrophotometry is more straightforward, cheaper, easier, and has excellent accuracy, precision, and detection limits (Gürkan & Altunay, 2018; Pourreza et al., 2012). This method has advantages over other instrumentation because it is simple, can measure small concentrations, the wavelength can be selected and is generally not too time-consuming (Porche, 2014). The spectrophotometric method can be used for qualitative examination of nitrite using sulfanilic acid and N-(1-naphthyl) ethylene dihydrochloride (NED) reagents which form a purple-red complex at a maximum wavelength of 540 nm (Jansen et al., 2018; Nerdy & De Lux Putra, 2018).

This research aims to determine the sodium nitrite content in dumplings available in online stores based on differences in storage. When consumers buy dumplings, they can be consumed endlessly when they are fresh, thereby causing the storage process to occur.

Methodology

The method used in this research refers to the method used by Ulfa (2020).

Material

The materials used are dumplings, sulfanilamide, sodium nitrite, n-1-naphthyl-ethylene diammonium dichloride (NED) solution, glacial acetic acid, distilled water, and filter paper. Meanwhile, the tools used in this research were several pieces of glassware and a Single-Beam UV-Vis Spectrophotometer (Thermo Scientific Genesys 20).

Procedure

Sample preparation

Ten grams of the ground sample was put into an Erlenmayer. Next, approximately 40 mL of distilled water heated at 80C was added to the sample and stirred. Then, hot water was added to the sample until the volume reached approximately 125 mL. The samples were kept in a water bath for 2 hours, shaking occasionally. Next, the sample was transferred into a 250 mL volumetric flask, rinsed with distilled water and cooled to room temperature. Next, the sample is diluted to the limit mark and filtered.

Determination of maximum wavelength

20 mL of 1 mg/L sodium nitrite standard solution was put into a 50 mL measuring flask, then 2.5 mL of sulfanilamide reagent was added and shaken. 2.5 mL of NED reagent was added to the solution, and then distilled water was added to the limit mark. The absorbance of the solution is measured with a wavelength between 400-800 nm, and the measurement results are recorded; then, a graph of the relationship between wavelength and absorbance is made.

Determination of the calibration curve

Pipette 0.25 mL of 1 mg/L Standard Solution, 0.5 mL, 0.75 mL, 1 mL, 1.25 mL, 2.5 mL; 5 mL, and 12.5 mL was then put into a different 25 mL measuring flask, 2.5 mL of sulfanilamide reagent was added, and 2.5 mL of NED reagent was added, obtaining a concentration of 0.01; 0.02; 0.03; 0.04; 0.05; 0.1; 0.2; and 0.5 mg/L, then distilled water was added to the limit mark, the absorbance of each standard solution was measured with a UV-Vis spectrophotometer at a previously determined maximum wavelength. The results of the measurements made a graph of the relationship between the concentration of the standard solution and the absorbance of the standard solution so that the line equation y=bx+a was obtained.

Measurement of sodium nitrite in sample

25 mL of sample solution was put into a 50 mL volumetric flask, 2.5 mL of sulfanilamide reagent was added and shaken, waited 5 minutes, then 2.5 mL of NED reagent was added and homogenised. Next, distilled water was added to the sample up to the mark, shaken, and left for 15 minutes until the sample was pink. Next, the sample can be measured using UV-Vis spectrophotometry at the maximum wavelength obtained in the previous stage.

Determination of sodium nitrite levels in samples

The sodium nitrite content in the sample is determined using Equation 1:

$$NaNO_2 \left(\frac{mg}{Kg}\right) = \frac{CxVxDF}{W} \quad (1)$$

where:
C : $NaNO_2$ concentration in the sample solution (mg/L)
V : Solution volume (L)
DF : Dilution Factor (10)
W : sample weight (kg)

Precision Determination

Precision in determining sodium nitrite in samples is based on the level of repeatability in duplicate, which is expressed as Relative Percent Different (RPD) with an acceptance limit of less than 5%(Standar Nasional Indonesia, 2004). The %RPD value is calculated based on Equation 2.

$$\%RPD = \left|\frac{x_1 - x_2}{\frac{x_1 - x_2}{2}}\right| x100\% \quad (2)$$

Determination of Estimation Uncertainty

The determination of uncertainty estimates is based on several components: sample volume, analytical balance, use of volumetric measuring instruments, calibration curves, precision, calculating combined uncertainty, and extended uncertainty. Figure 1 shows the fishbone on uncertainty estimates in determining sodium nitrite in samples.

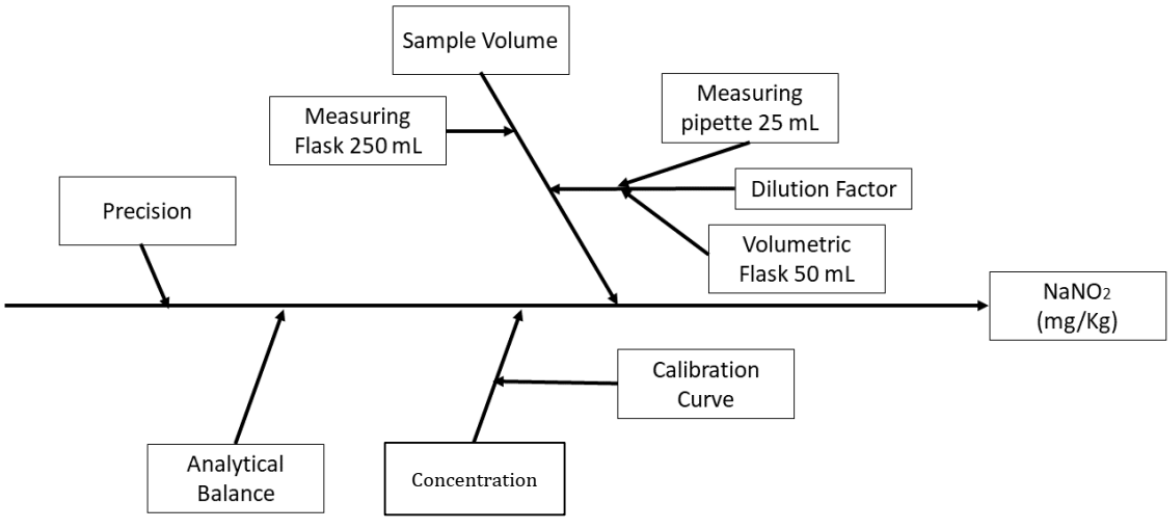

Figure 1. Fish bones on uncertainty estimates in sodium nitrite determination.

Result and Discussion

The first thing to be done before testing a sample is determining the maximum wavelength. In this study, sodium nitrite in the sample was determined using UV-Vis spectrophotometry using the maximum wavelength. The maximum wavelength was determined by 540 nm (Figure 2). This stage needs to be carried out to see the sensitivity of the detection tool to the measurement results. The results obtained are not much different from those of previous researchers (Table 1.) This shows that the results of this test are worth continuing.

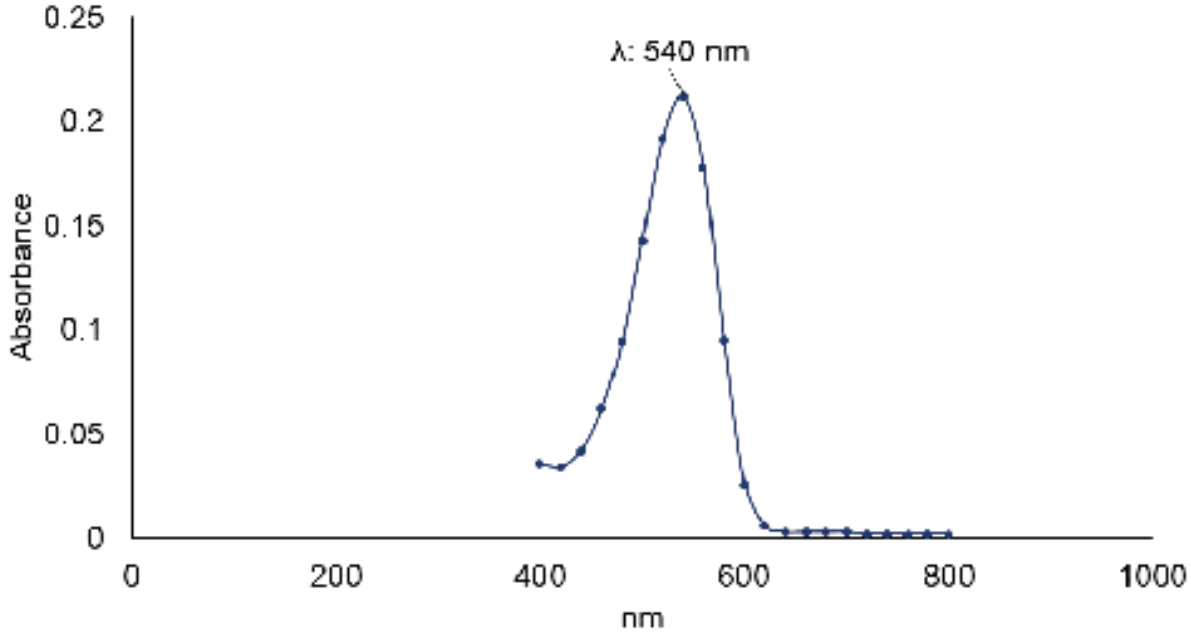

Figure 2. Maximum Wavelength for Determination of Sodium Nitrite in samples

Table 1. Determination of maximum wavelength

No	Wavelength Maximum (nm)	Sample	References
1	540	Cured Meat	(Mohamed et al., 2008)
2	540	Ham Pate	(Reis et al., 2009)
3	540	Bahan Makanan	(Pandurangappa & Venkataramanappa, 2011)
4	540	Captopril	(Porche, 2014)
5	536	Corned Beef And Smoked Beef	(Jansen et al., 2018)
6	545	Daging Sapi Olahan	(Maria Ulfa & Lutfiana, 2020)
7	540	Market Sausages	(Yuan et al., 2021)
8	541	Daging Burger Sapi	(Saad et al., 2021)
9	520	Meat	(Guembe-García et al., 2022)
10	540	Meat Sausages	(Almeida et al., 2022)
11	538	Vienna Chicken Sausages	(Tantinantrakun et al., 2023)
12	540	Dumpling	This Work

Determination of Calibration Curves, detection limits and quantification limits

The calibration curve converts the results of sample absorbance measurements into concentration form by interpolating the linear regression equation, y=mx+c. The calibration curve requirement can be used in data analysis if the calibration curve has an R2 value > 0.990 (Miller & Miller, 2010). The results of this study show an R2 value of 0.9977 (Table 2). These results indicate a strong relationship between the concentration of the standard solution and the resulting absorbance, namely 99.7%.

Table 2. Linearity of Calibration Curve

Regression Equations	R^2	r	Slope	Intercept	DL	QL
y = 0.6943x + 0.0001	0.9977	0.9989	0.6943	0.0001	0.0043	0.0144

According to ICH (2022), to declare the sample measurement results good, they can be compared with the detection limit (DL) and quantification limit (QL) values. The Detection Limit and Quantification Limit are the minimum concentrations at which an analyte can be reliably detected or measured. A signal-to-noise ratio of 3:1 is generally considered acceptable for estimating detection limits. For quantitation limits, a minimum ratio of 10:1 is considered acceptable.

Determination of detection limits using Equation 3.

$$DL = \frac{3.3\sigma}{S} \quad (3)$$

Meanwhile, the quantification limit is determined by Equation 4.

$$QL = \frac{10\sigma}{S} \quad (4)$$

Where σ is the standard deviation of the response, while S is the slope of the calibration curve.

Table 2 shows that the DL and QL values are 0.0043 mg/L and 0.0144 mg/L, respectively. This means that the minor limit for an analyte that can be detected is 0.0043

mg/L, and the limit for an analyte that can be measured is 0.0144 mg/L.

Quantitative Analysis of Sodium Nitrite in Samples

The determination of sodium nitrite in dumpling samples is based on the reaction between sodium nitrite with sulfanilamide and NED reagents. The sodium nitrite in the sample will be converted into a nitrite ion and then react with the sulfanilamide compound, which has the $-NH_2$ group. In acidic conditions, this reaction produces benzene diazonium ions. Next, the ion will be coupled with the NED reagent to form an azo compound marked with a purple-red colour in the solution (Budi & Havizul, 2022). The reaction mechanism for determining sodium nitrite is presented in Figure 3.

Table 3 presents the results of determining sodium nitrite in the sample. These results show that the sodium nitrite content in the sample is still below the limit determined by the National Agency of Drug and Food Control of the Republic of Indonesia No. 11/2019, namely 30 mg/Kg in both the fresh and after 24 Hours of Storage categories. This means that the sample is still suitable for consumption by the wider community, and the product is suitable for circulation on the market.

Table 3. Sodium Nitrite in Samples

No	Category	Sample Code	[mg/L]	mg/Kg	Threshold (mg/Kg) *
1	Fresh	MT	0.198	9.890	30
2		CD	0.311	15.545	
1	After 24 Hours of Storage	MT	0.137	6.862	
2		CD	0.264	13.209	

*) National Agency of Drug and Food Control the Republic of Indonesia No. 11/2019

Determination of Precision

The research results show that the %RPD value exceeds the acceptance requirement of <5% for fresh and After 24 Hours of Storage category samples. This means that testing sodium nitrite on samples using this method does not meet the acceptance requirements. However, when compared with the 0.5*CV Horwitz value, the %RPD value still meets the acceptance requirements, namely the %RPD < 0.5*CV Horwitz value. So, this test can be declared to meet the acceptance requirements.

Table 4. Determination of Precision

No	Category	Sample Code	SD	%RPD	CV Horwitz	0.5*(CV Horwitz)
1	Fresh	MT	0.014	7.030	20.420	10.210
2		CD	0.022	6.954	19.080	9.540
1	After 24 Hours of Storage	MT	0.012	8.382	21.570	10.790
2		CD	0.005	1.921	19.550	9.770

The test results also follow previous research on the determination of sodium nitrite in food samples using the IC-CD method (Coviello et al., 2020), UV- VIS-NIR Scanning Spectrophotometer (Pandurangappa & Venkataramanappa, 2011), UV-Vis Spectrophotometer (Rashid, 2006; Reis et al., 2009), ion-exchange chromatography (Mazumdar et al., 2022), and UV/Vis microplate spectrophotometer (Thipwimonmas et al., 2021).

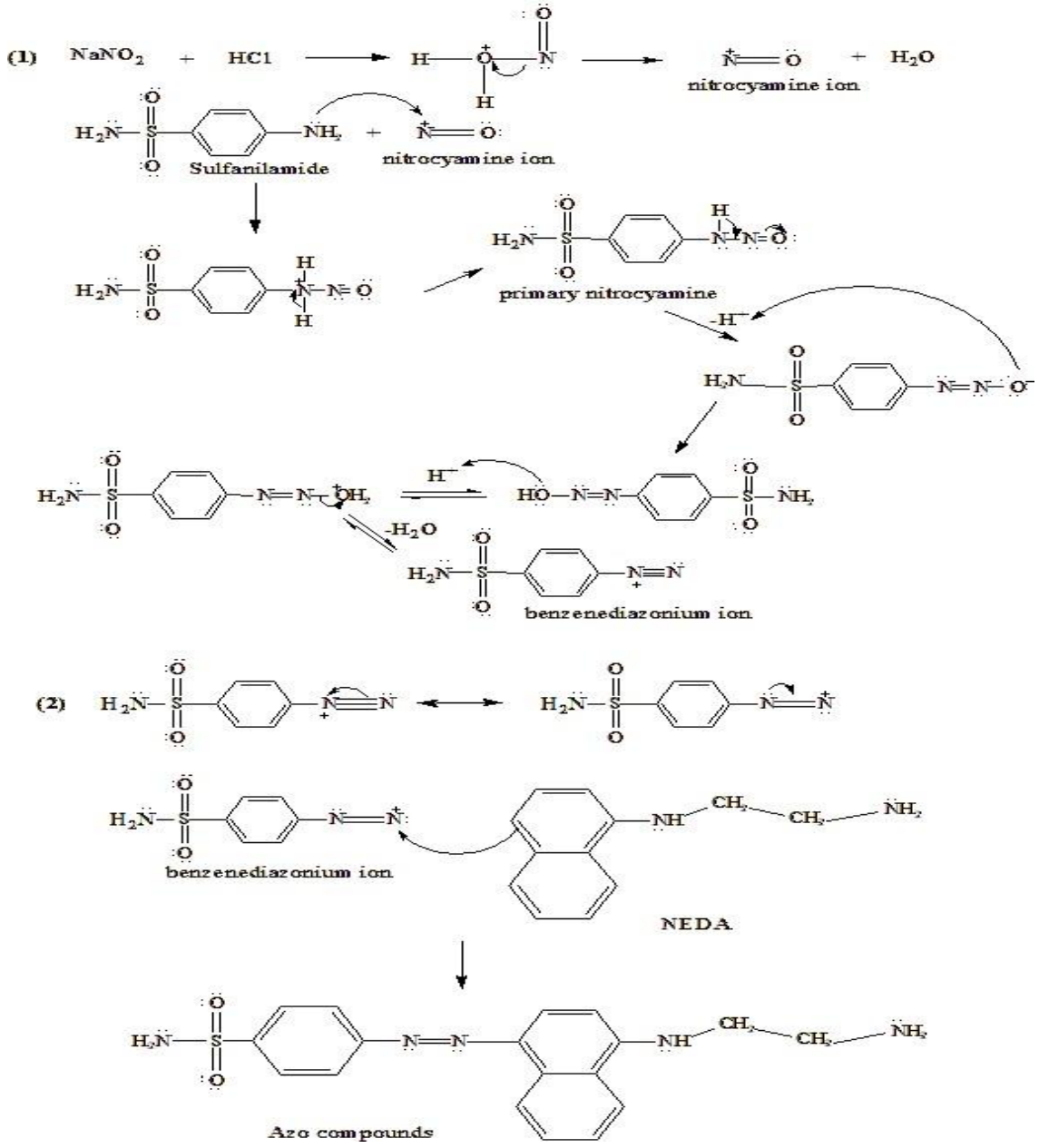

Figure 3. Determination reaction of sodium nitrite (Budi & Havizul, 2022)

Determination of Estimation Uncertainty

Table 5 shows the results of uncertainty estimation calculations in determining sodium nitrite in samples using UV-Vis spectrophotometry at a maximum wavelength of 540 nm.

Table 5. Determination of uncertainty estimates in the sample

No	Category	Sample Code	mg/Kg	Expanded Uncertainty (μ)
1	Fresh	MT	9.890	1.072
2		CD	15.545	1.681
1	After 24 Hours of Storage	MT	6.862	0.862
2		CD	13.209	0.688

Table 5 shows the estimated values of expanded uncertainty (μ) in the determination of sodium nitrite in dumpling samples. The acceptance requirement for the estimated uncertainty is less than 20-30% of the analyte level (Kristiansen & Christensen, 1998; Ramsey et al., 2019). This uncertainty estimate value determines the acceptability limits of data from traceable sources. So it can be said that the value of sodium nitrite content in dumpling samples for the fresh category with sample codes MT and CD is respectively 9,890 ± 1,072 mg/Kg and 15,545 ± 1,681 mg/Kg. Meanwhile, for samples after the storage process for 24 hours for sample codes MT and CD, respectively, it was 6,862 ± 0.862 mg/Kg and 13,209 ± 0.688 mg/Kg.

Conclusion

The research results show that the sodium nitrite content is still below the threshold (<30 mg/K). Meanwhile, precision still meets the acceptance requirements. The estimated uncertainty value is also below the acceptance requirement of 30% of the level.

References

Almeida, G. A., Demuner, A. J., Blank, D. E., Cerceau, C. I., Sousa, B. L., Demuner, I. F., Coura, M. R., Firmino, M. J. M., & Santos, M. H. (2022). An Alternative and Fast Method of Nitrite Determination in Meat Sausages Using the PhotoMetrix® Smartphone Applicative for Digitized Image Processing. Open Access Library Journal, 9, 1–10. https://doi.org/10.4236/oalib.1108689

Peraturan Badan Pengawas Obat Dan Makanan tentang Bahan Tambahan Pangan Nomor 11 Tahun 2019 Tentang Bahan Tambahan Pangan, Badan Pengawas Obat dan Makanan Republik Indonesia 1 (2019).

Budi, F. S., & Havizul, H. (2022). Pengembangan Metode Penentuan Nitrit Dan Nitrat Dalam Daging Segar Dan Produk Daging Olahan Secara Spektrofotometri Uv-Vis. ILTEK : Jurnal Teknologi, 17(02), 120–129. https://doi.org/10.47398/iltek.v17i02.21

Coviello, D., Pascale, R., Ciriello, R., Salvi, A. M., Guerrieri, A., Contursi, M., Scrano, L., Bufo, S. A., Cataldi, T. R. I., & Bianco, G. (2020). Validation Of An Analytical Method For Nitrite And Nitrate Determination In Meat Foods For Infants By Ion Chromatography With Conductivity Detection. Foods, 9(1238). https://doi.org/10.3390/foods9091238

Guembe-García, M., González-Ceballos, L., Arnaiz, A., Fernández-Muiño, M. A., Sancho, M. T., Osés, S. M., Ibeas, S., Rovira, J., Melero, B., Represa, C., García, J. M., & Vallejos, S. (2022). Easy Nitrite Analysis of Processed Meat with Colorimetric Polymer Sensors and a Smartphone App. ACS Appl. Mater. Interfaces, 14(32), 37051–37058. https://doi.org/10.1021/acsami.2c09467

Gürkan, R., & Altunay, N. (2018). Preconcentration And Indirect Quantification Of Trace Nitrite, Nitrate And Total Nitrite In Selected Beverage And Milk Samples Using Ion-Pairing Cloud-Point Extraction With Acridine Orange. Journal of Food Composition and Analysis, 69, 129–139. https://doi.org/10.1016/j.jfca.2018.03.002

Hardiman, C. I. (2017). Resep Pilihan Ny. Liem: Dim Sum Istimewa (1st ed.). Gramedia Pustaka Utama.

ICH. (2022). ICH Guideline Q2(R2) on Validation of Analytical Procedures (pp. 1–39). https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r2-validation-analytical-procedures-step-2b_en.pdf

Jansen, S., Dera, R. T. S., Ruth, M. S. M., Syofyasti, M. N., Muchlisyam, & Silalahi

Yosy, C. E. (2018). Analysis of Nitrite and Nitrate in the Corned Beef and Smoked Beef by Using Visible Spectrophotometry Method. IOP Conference Series: Earth and Environmental Science, 205, 1–7. https://doi.org/10.1088/1755-1315/205/1/012039

Katzung, B. G. (2004). Farmakologi Dasar dan Klinik (3rd ed.). Salemba Medika.

Kristiansen, J., & Christensen, J. M. (1998). Traceability and Uncertainty in Analytical Measurements. Annals of Clinical Biochemistry, 35, 371–379. https://doi.org/10.1177/000456329803500305

Maria Ulfa, A., & Lutfiana, A. (2020). Analisis Kandungan Natrium Nitrit Pada Daging Sapi Olahan Dengan Spektrofotometri Uv-Vis. Jurnal Analis Farmasi, 5(2), 73–80.

Mazumdar, R. M., Sharif, M., Khan, T. A., Rahman, M. M., & Abdullah, A. T. M. (2022). Simultaneous Determination of Nitrite and Nitrate in Meat and Meat Products Using Ion-Exchange Chromatography. Food Research, 6(3), 145–152. https://doi.org/10.26656/fr.2017.6(3).339

Miller, J. N., & Miller, J. C. (2010). Statistics and Chemometrics for Analytical Chemistry (Sixth). Pearson Education Limited. https://doi.org/10.7861/clinmedicine.14-6-677

Mohamed, A. A., Mubarak, A. T., Fawy, K. F., & El-Shahat, M. F. (2008). Modification of AOAC Method 973.31 for Determination of Nitrite in Cured Meats. Journal of AOAC International, 91(4), 820–827. https://doi.org/10.1093/jaoac/91.4.820

Nerdy, N., & De Lux Putra, E. (2018). Spectrophotometric Method for Determination of Nitrite and Nitrate Levels in Broccoli and Cauliflower with Different Fertilization Treatment. Oriental Journal of Chemistry, 34(6), 2983–2991. https://doi.org/10.13005/ojc/340639

Nessianti, A., & Soeyono, R. D. (2015). Pengaruh Penambahan Puree Labu Siam (Sechium Edule) Terhadap Sifat Organoleptik Siomay Ikan Tenggiri (Scomberomorus Commersoni). E-Jurnal Boga, 4(3), 79–84.

Pandurangappa, M., & Venkataramanappa, Y. (2011). Quantification of Nitrite/Nitrate in Food Stuff Samples Using 2-Aminobenzoic Acid as a New Amine in Diazocoupling Reaction. Food Analytical Methods, 4(1), 90–99. https://doi.org/10.1007/s12161-010-9138-4

Porche, M. (2014). Spectrophotometric Determination Of Nitrite By Derivation With Captopril. Miami University.

Pourreza, N., Fat'hi, M. R., & Hatami, A. (2012). Indirect Cloud Point Extraction and Spectrophotometric Determination of Nitrite in Water and Meat Products. Microchemical Journal, 104, 22–25. https://doi.org/10.1016/j.microc.2012.03.026

Pulung, A. F. (2019). Dampak Pengawet Nitrit Pada Daging Olahan Sosis Terhadap Kesehatan Manusia. Deepublish.

Ramsey, M. ., Ellison, S. L. ., & Rostron, P. (2019). Measurement Uncertainty Arising from Sampling: A Guide to Methods and Approaches (2nd ed.). http://www.eurachem.org/guides/pdf/UfS_2007.pdf

Rashid, C. M. (2006). Spectrophotometric Determination of Nitrite in Curing Meat Samples Using Diazo-Coupling Reaction. Tikrit Journal of Pure Science, 11(2), 183–186. www.codexalimentarius.net

Reis, P. B., Ramos, R. M., De Souza, L. F., & Cançado, S. D. V. (2009). Validation of Spectrophotometric Method to Detect and Quantify Nitrite in Ham Pate. Brazilian Journal of Pharmaceutical Sciences, 45(1), 49–55.

https://doi.org/10.1590/S1984-82502009000100006

Saad, A. A., Dalming, T., Nurdin, A. P., & Basri, Z. (2021). Penetapan Kadar Natrium Nitrit Pada Daging Asap dan daging Burger Sapi yang Beredar di Kabupaten Gowa Dengan Metode Spektrofotometri UV-Vis. *Jurnal Farmasi Pelamonia*, *1*(1), 1–4.

Standar Nasional Indonesia. (2004). *Air dan air limbah – Bagian 9: Cara Uji Nitrit (NO2-N) Secara Spektrofotometri* (SNI 06-6989.9-2004). Badan Standardisasi Nasional. sainstkim.teknik.ub.ac.id

Tantinantrakun, A., Thompson, A. K., Terdwongworakul, A., & Teerachaichayut, S. (2023). Assessment of Nitrite Content in Vienna Chicken Sausages Using Near-Infrared Hyperspectral Imaging. *Foods*, *12*(2793), 1–11. https://doi.org/10.3390/foods12142793

Thipwimonmas, Y., Jaidam, J., Samoson, K., Khunseeraksa, V., Phonchai, A., Thiangchanya, A., Chang, K. H., Abdullah, A. F. L., & Limbut, W. (2021). A Simple and Rapid Spectrophotometric Method for Nitrite Detection in Small Sample Volumes. *Chemosensors*, *9*(7), 1–11. https://doi.org/10.3390/chemosensors9070161

Yuan, L., Zhang, J., Wu, X., Li, N., Liu, H., & He, L. (2021). The Determination of Nitrite Content in Market Sausages. *IOP Conference Series: Earth and Environmental Science*, *631*(1). https://doi.org/10.1088/1755-1315/631/1/012036