ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 26, No. 1 (2025) 49 – 57

http://doi.org/10.21743/pjaec/2025.06.05

Application of FTIR and NIR Spectroscopy Coupled with Chemometrics for Determination of Monosodium Glutamate Level in Balado Seasoning

Lestyo Wulandari[1]*, Linda Devitasari[1] and Indah Purnama Sary[1]

[1]*Faculty of Pharmacy, University of Jember, Kalimantan Street 37 Jember, 68121, Indonesia.*

*Corresponding author Email: wulan.farmasi@unej.ac.id

Received 07 November 2023, Revised 23 August 2024, Accepted 16 October 2024

Academic Editor: Sarfaraz Ahmed Mahesar

Abstract

Balado seasoning is a spicy powder containing chili, spices and monosodium glutamate (MSG). MSG is a natural monosodium salt in the form of L-glutamic acid, used as a savory taste in food. This study aims to determine MSG in Balado seasoning using near-infrared (NIR) and Fourier transform infrared (FTIR) spectroscopy methods combined with chemometrics. The comparison method used was the thin layer chromatography (TLC)-Densitometry method. Support Vector Regression (SVR), Principal Component Regression (PCR) and Partial Least Square (PLS) were used to form a calibration model. Then the selected calibration model was validated by the leave one out cross validation (LOOCV) and external validation methods. Based on the research result, the PLS model is the selected calibration model with both the NIR and FTIR methods which gives the best results with an R^2 value > 0.91. The results of the LOOCV validation have met the parameter values of $R^2 > 0.91$, and the external validation result using the NIR and FTIR methods yielded R^2 values of 0.999 and 0.997 and RMSEP value of 0.093 and 0.284, respectively. The concentration of MSG in commercial samples obtained by TLC Densitometry as a reference method were 1.314%, 1.449%, 5.836%, and 9.672%. The results of the analysis of MSG concentration as measured by the NIR and FTIR spectroscopy compared to TLC Densitometry did not give a significant difference.

Keywords: Monosodium glutamate, NIR spectroscopy, FTIR spectroscopy, Chemometrics, Balado seasoning, TLC Densitometry

Introduction

Balado seasoning is one of the instant spices that give food a savory or umami taste [1]. The ingredients of Balado seasoning are red chili powder, garlic powder, pepper powder, salt, sugar, and monosodium glutamate (MSG). MSG is the sodium salt of glutamic acid in the form of L-glutamic acid. Glutamic acid is a non-essential amino acid that the body can produce. The main compounds in MSG are glutamate (78.2%), sodium (12.2%), and H_2O (9.6%). MSG has the characteristics of white crystals that are very soluble in water, practically odorless, and has a meat-like taste [2].

Based on the Food and Drug Administration (FDA) and the Federation of American Societies for Experimental Biology (FASEB), the recommended level of MSG usage is 0.1%-0.8% or equivalent to 1–8 g/kg of food, either without the addition of other flavorings or in combination with others [3,4].

Due to the absence of MSG levels on the packaging labels for instant seasoning products, consumers tend to consume MSG in excess. The FASEB reports that excessive consumption of MSG in some people can cause MSG Symptom Complex. Several studies have reported that the toxic effects of consuming high levels of MSG can affect the function of specific organs, such as liver and kidney damage, impaired embryonic development, and reproductive dysfunction.

Previously reported determination of MSG levels using spectrofluorometric method [5], high performance liquid chromatography with diode array detector [6] and fluorescence detector [7], gas chromatography [8], and thin layer chromatography [9,10]. These methods have drawbacks, including taking a long time, and the costs used are relatively not cheap. Infrared (IR) spectroscopy is a non-destructive method with many advantages, such as not requiring chemical reagents, not causing pollution, and being able to analyze quickly [11]. IR spectra are generally difficult to interpret because the spectra data are very complicated, so the IR spectroscopy method (NIR and FTIR) must be combined with multivariate statistical methods [12]. Chemometrics is a multivariate statistical method that makes it easier to analyze spectra, so by combining the IR spectroscopy method with chemometrics, it is hoped that more efficient assay results will be obtained on samples because it is performed quickly and economically. The application of chemometrics using direct spectroscopy, especially IR spectroscopy, has been reported to be a quality control tool in the pharmaceutical industry [13] and as quality inspection for agricultural and food products [14]. There is no report of quantitative analysis of MSG in the seasoning products using IR spectroscopy. A qualitative study of the pattern of FTIR spectra in several seasoning products has been reported [15].

Based on considerations of increasing consumption of Balado seasoning products, health aspects, and more straightforward and more valid alternative methods, this study aims to determine the NIR-FTIR chemometric method for determining the levels of MSG in the Balado seasoning sample and whether there is a significant difference between the MSG levels determined by the NIR-FTIR chemometric method in comparison to TLC densitometry method.

Materials and Methods

Materials

The reagents used in the research were sterile distilled water, pro-analytical reagents (Merck), i.e., chloroform, methanol, formic acid, ninhydrin, and acetone. The materials used were TLC plate silica F254 (Merck) and Whatman filter paper (Merck), the standard MSG with a purity of 99.46% obtained from PT. SASA Industry, Indonesia. The ingredients of Balado seasoning were red chili powder, pepper powder, garlic powder, granulated sugar, and salt. The commercial samples of the balado seasoning were bought at minimarkets and traditional markets in Jember, Indonesia.

Instruments

The instruments used in this research were an NIR spectrophotometer (Brimrose corporation Luminar 3070) equipped with The Unscrambler X 10.2 (Camo) software, an FTIR spectrometer (Alpha FTIR Spectrometer from Bruker) analytical balance, mortar and pestle, Camag densitometer with winCATS software, SPSS version 22, ultrasonicator, TLC chamber, capillary tube, and glassware.

Simulated Sample Preparation

According to the composition claim of the commercial sample, the simulated sample was made consisted of a mixture of red chili

powder (70%), garlic powder (12%), pepper powder (4.0%), salt (7.0%), and sugar 7.0% g). It is prepared by weighing a certain amount of each ingredient composition, mixing it until homogeneous, and then adding it with MSG at various concentrations (0.05%-16.0%). The simulated samples devided in to the training set and test set samples. Training set samples are samples used to form a chemometric calibration model. The training set samples consist of 36 simulated samples with MSG concentration of 0.05%-16.0%. The test set is a sample to evaluate the reliability of the model formed. The sample test set in this study was 12 simulated samples with an MSG concentration of 0.15% - 14.5% [13].

Sample Scanning Using NIR and FTIR Spectroscopy

The instrument used for scanning the sample was the Brimrose NIR Luminar 3070. The simulated sample was placed on the sample plate while pressed slowly until the powder compressed and filled the compartment homogenously. The NIR spectra were scanned at a range of 850-2500 nm. Each scanning of the simulated sample and ingredients of Balado seasoning were replicated three times, and each replication was subjected to three shots.

The FTIR spectra of the samples were measured at room temperature (25±0.5 °C) and dry atmosphere using Bruker FTIR spectrophotometer with ATR sampling accessory. Approximately 1.0 g of sample powder was put in and covered the sampling accessory. The spectrum was obtained from the average of five readings and recorded from 650 to 4,000 cm^{-1} at a resolution of 4 cm^{-1} and 32 scans. The ATR plate was cleaned carefully by wiping it with 70% isopropyl, drying it with soft tissue, and allowing the ATR plate to dry before filling it with the following sample. This ATR scanning was done in three replications. The data were exported to unscrambler format for further processing.

Model Calibration and Validation

The formation of the calibration model from the obtained NIR spectroscopic measurement data was processed using The Unscrambler software. Partial Least Square (PLS), Principal Component Regression (PCR), and Support Vector Regression (SVR) were used as the chemometrics calibration model. Calibration models were evaluated using several parameters, including the value of R^2 (coefficient of determination), the value of RMSEC (Root Mean Square Error of Calibration), and the value of RMSECV (Root Mean Square Error Cross of Validation). The selection of the spectrum data set was based on the best predictive ability with the closing value of R^2 to one and the smaller value of RMSEC and RMSECV. The validation of the chemometric model used the leave one out cross validation (LOOCV) method and external validation. External validation was performed using an independent sample outside the training set sample [13].

Determination of MSG Levels Using the TLC-Densitometry method

The method of determining MSG levels in this study used the TLC-Densitometry method based on Krishna et al.'s research [10] with several modifications and optimization of the TLC analytical conditions [16]. The MSG standard solutions were prepared having concentration of 50, 100, 200, 300, 400, 500, and 800 µg/mL. The eluent used was the mixture of chloroform, methanol, and formic acid (4:6:1 v/v). After saturation, the plate was put in the chamber and eluted by the eluent for fifteen minutes, and then removed, dried, and derivatized with 1% ninhydrin solution in acetone. After the plate dried, scanning was carried out using a densitometer at a wavelength of 485 nm.

Commercial Sample Application

The selected commercial samples were scanned using NIR spectroscopy, and then the absorbance spectrum data obtained were analyzed using the best calibration model that had been established. The TLC-Densitometry method was used as a comparison method. The MSG content results obtained from the NIR-Chemometric method were compared with the TLC Densitometry method and then analyzed using the paired sample t-test. A result is considered not statistically significant when $p > 0.05$ in two-tailed testing at $\alpha = 0.05$ [17,18].

Results and Discussion

The NIR and FTIR spectra of the MSG standard, the ingredient samples (red chili powder, garlic powder, pepper powder, salt, and sugar), the training sets and commercial samples of balado seasoning can be seen in Fig. 1 and 2. NIR spectra are more accessible to distinguish compared to FTIR spectra, which have many fingerprint peaks. The MSG spectra differ from the ingredients of sample simulation, the simulated samples, and the commercial samples. However, the simulation training set sample and the actual sample of Balado seasoning have similar spectral patterns. MSG provides a savory taste formed by amino acids (glutamic acid) or proteins. Proteins are polyamino acids that have R-NH (amine) functional groups. The absorbance peak can identify the amine functional group in the FTIR spectrum at wave numbers 2,250-4,000 cm^{-1}. Glutamic acid also has carboxylic acid that can be shown by the presence of absorbance peak at wave numbers 2150-3120 cm^{-1}. In the NIR spectrum, the amine (R-NH) group is shown in the absorbance peak at 1500-1536 nm, N–H and O–H combination bands at 2150-2238 nm wavelength and the carboxylic acid group at 1908–1912 nm [19].

This study's calibration models used seven latent variables in the PLS model, six principal components for the PCR model, and the default kernel function and trade-off parameter, i.e., 0.5, were used in SVM models. The PLS, PCR, and SVR yielded good results with R^2 of more than 0.959. A model was good if it had an R^2 value > 0.91 [18]. The RMSEC and RMSECV values are the error values between the reference and predictions in the model. The smaller the RMSEC and RMSECV values, the better the model is established [20]. The results of the PLS, PCR, and SVR calibration models with the NIR and FTIR methods can be seen in Table 1 and 2.

Table 1. **Results of the training set sample calibration model using the NIR spectra.**

Model	Process	Slope	RMSE	R^2
PLS	calibration	0.983	0.627	0.983
	validation	0.981	0.711	0.978
PCR	calibration	0.962	0.940	0.962
	validation	0.958	0.978	0.959
SVR	calibration	-	0.740	0.978
	validation	-	0.934	0.963

Table 2. **Results of the training set sample calibration model using the FTIR spectra.**

Model	Process	Slope	RMSE	R^2
PLS	calibration	0.987	0.545	0.987
	validation	0.985	0.600	0.985
PCR	calibration	0.922	1.344	0.922
	validation	0.912	1.420	0.914
SVR	calibration	-	0.732	0.977
	validation	-	0.773	0.974

NIR spectra do not have many sharp peaks, so compared to FTIR spectra, NIR spectra are pretty smooth. When the spectra have a good coefficient of variation below 30% [21], they can be used to generate a calibration model. FTIR spectra have sharp peaks that must be smoothed by preprocessing before developing a calibration model.

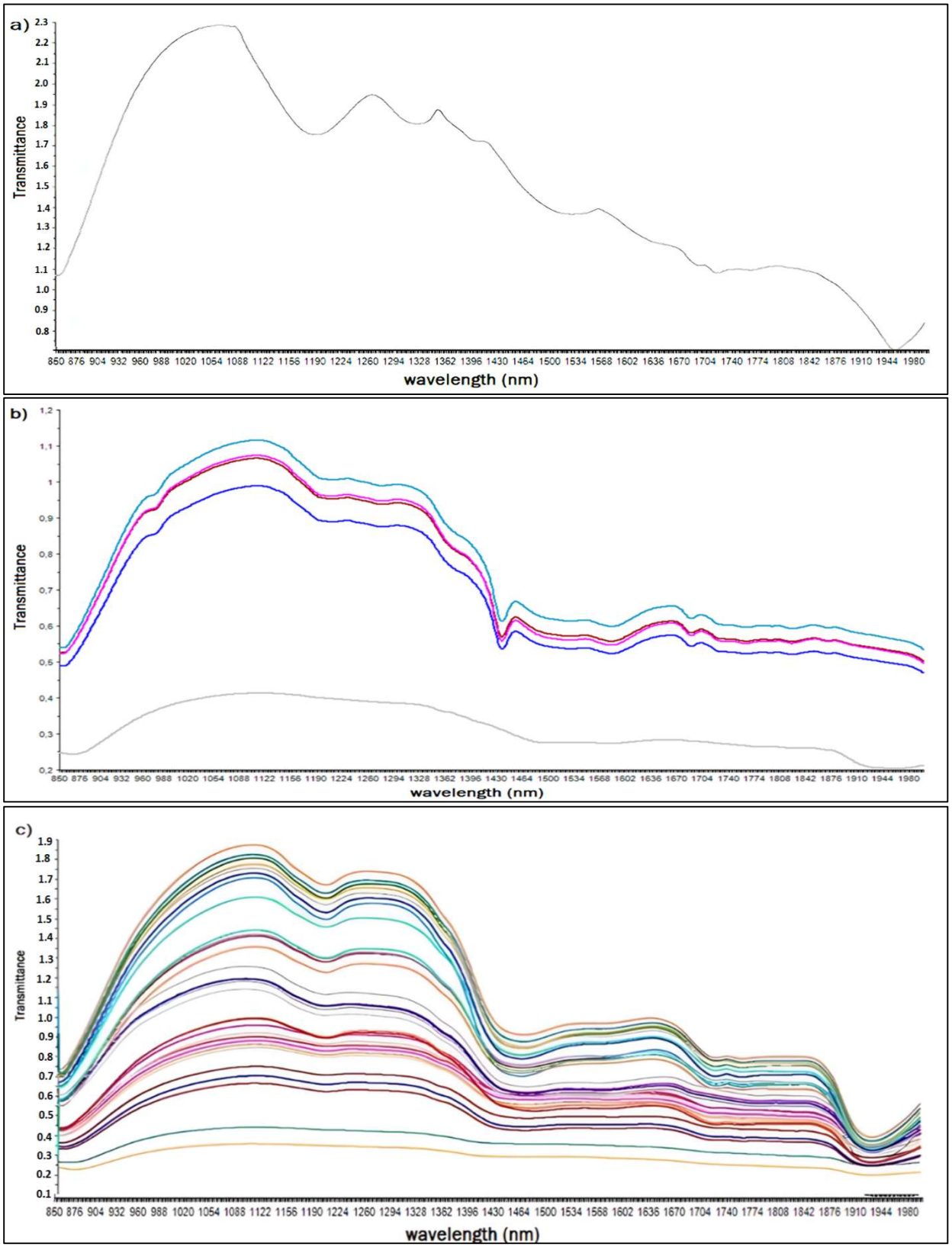

Figure 1. **a) NIR spectra of MSG; b) NIR spectra of ingredient samples (red chili powder, garlic powder, pepper powder, salt, and sugar; c) NIR spectra of the training sets and commercial samples of balado seasoning**

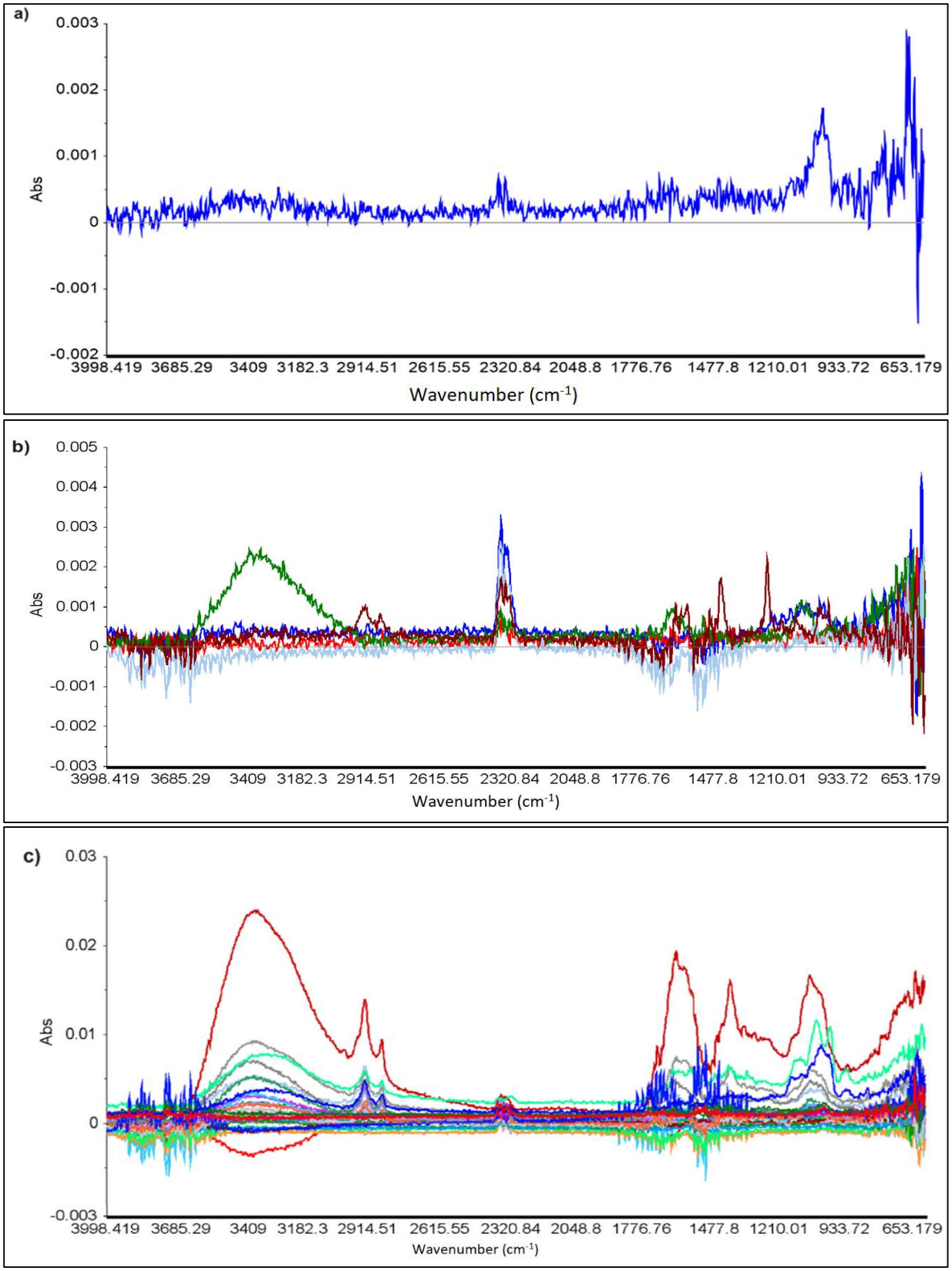

Figure 2. **a) FTIR spectra of MSG; b) FTIR spectra of ingredient samples (red chili powder, garlic powder, pepper powder, salt, and sugar; c) FTIR spectra of the training sets and commercial samples of balado seasoning**

The results of the formation of the calibration model indicate that the three calibration models formed, namely, PLS, PCR, and SVR, have met the criteria for a good calibration model where the best model was the PLS model with the highest R^2 value, the smallest RMSEC value. The LOOCV and external validation were used to evaluate the PLS model.

The results of the validation of the calibration model for each method can be seen in Table 3. LOOCV was performed by removing one of the training set samples, and the remaining samples were used to form a new PLS model [22]. Table 3 shows that the R^2 value generated in the LOOCV has a good result based on correlation coefficient values more than 0.966 and the development of the RMSECV values was less than 0.888, which indicates the PLS model has a small error value.

Tabel 3. **LOOCV results of the PLS model NIR and FTIR spectra.**

Spectra	MSG (%) concent-ration of removed sample	R^2 Calibration	R^2 Validation	RMSEC	RMSECV
NIR	0.05	0.970	0.966	0.831	0.888
	5.00	0.983	0.979	0.628	0.710
	8.75	0.970	0.967	0.837	0.878
FTIR	0.60	0.987	0.984	0.545	0.618
	9.00	0.986	0.982	0.573	0.647
	16.0	0.987	0.983	0.518	0.591

The external validation used independent test set samples (12 samples) to predict the concentration of the sample from the selected calibration model. The results of the external validation method of the PLS model with the NIR spectra have R^2 value and RMSEP value of 0.999 and 0.093, respectively (Fig. 3). The results of the external validation method of the PLS model with the FTIR spectra have an R^2 value and RMSEP value of 0.997 and 0.284, respectively (Fig. 4).

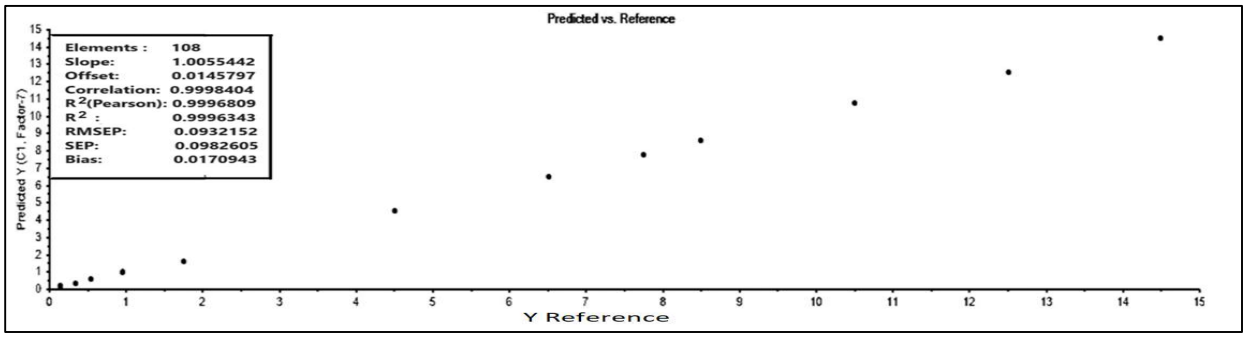

Figure 3. **Results of the external validation method of PLS model with NIR spectra**

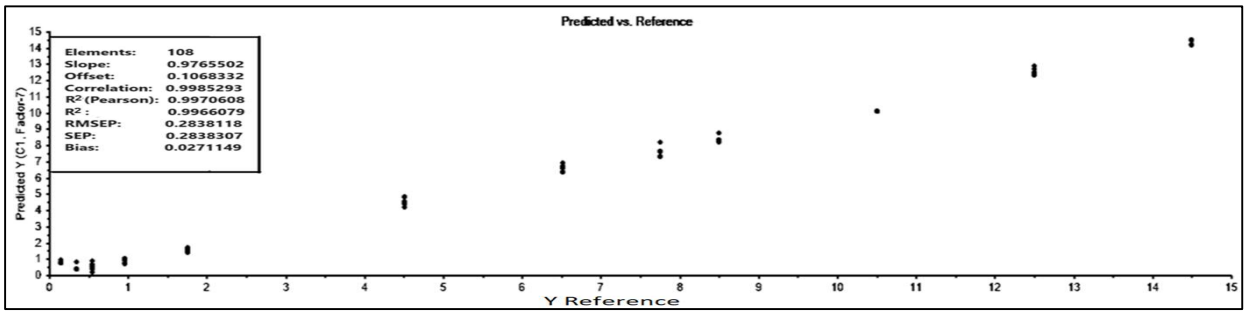

Figure 4. **Results of the external validation method of the FTIR spectra**

The PLS calibration model was applied to the determination of MSG levels in commercial samples. The results of comparison method for the determination of MSG levels in commercial samples using PLS-NIR and TLC densitometry can be seen in Table 4. Based on the FDA and the FASEB, recommendations for the use of MSG in food are 0.1%-0.8% or equivalent to 1–8 grams/kg of food, either without the addition of flavoring other flavors or in combination with other flavorings [4]. One of the commercial samples has MSG levels exceeding 1%.

Tabel 4. **Result of MSG level in commercial samples.**

Code of sample	Concentration of MSG (% w/w ± SD, n=3)		
	PLS - FTIR	PLS - NIR	TLC Densitometry method
A	9.665 ± 0.009	9.826 ± 0.020	9.672 ± 0.078
B	5.805 ± 0.012	5.615 ± 0.010	5.836 ± 0.139
C	1.760 ± 0.042	1.416 ± 0.009	1.449 ± 0.035
D	1.308 ± 0.073	1.201 ± 0.012	1.314 ± 0.022

The results of the assay of the samples were then analyzed by using the paired sample t test. Before performing the paired sample t-test, a normality test was performed first to determine whether the data distribution was normal or not. The data requirements were normally distributed if the resulting significance value was > 0.05. In the normality test, a significance value of > 0.05 was obtained, which means that the data from the two methods used were normally distributed. If the normality test conditions have been fulfilled, it was continued with the paired sample t-test analysis. Based on the results of the paired sample t-test analysis, the NIR and TLC densitometry methods had a significance value of 0.741 (>0.05). Meanwhile, the FTIR and TLC densitometry methods showed a significance value of 0.339 (> 0.05). This means that there is no significant difference between the results obtained from the NIR method and the FTIR method with the TLC-Densitometry method. So it can be concluded that the NIR-Chemometric and FTIR methods can be applied to determine the MSG content of the Balado seasoning.

Conclusion

The NIR and FTIR spectroscopy combined with chemometrics can determine MSG in Balado seasoning. The best calibration model was PLS, which has the best R^2 value and the RMSE value. The results of the LOOCV validation have an R^2 value of more than 0.91 and an RMSECV value of less than 0.888. The external validation result using the NIR and FTIR methods yielded R^2 values of 0.999 and 0.997 and RMSEP values of 0.093 and 0.284, respectively. The concentration of MSG in commercial samples obtained by TLC Densitometry as a reference method were 1.314%, 1,449%, 5,836%, and 9.672%. The comparison result of MSG content determined by PLS of NIR and FTIR and the TLC-densitometry methods showed no significant differences. This alternative method is rapid, simple, precise, and accurate.

Reference

1. Ernawati, H. T. Palupi and E. A. Alfianto, *J. Agromix*, 9 (2018) 57. https://doi.org/10.35891/agx.v9i2.1262
2. K Wijayasekara and J.Wansapala, *Int. J. Food Sci. Nutr*., 2 (2017) 132. https://www.foodsciencejournal.com/assets/archives/2017/vol2issue3/2-3-24-214.pdf
3. FASEB. *Analysis of Adverse Reaction to Monosodium Glutamate (MSG)*, Life Science Research Office: US, (1995) 2891. https://doi.org/10.1093/jn/125.11.2891S

4. H. D. B Maluly, A. P. A. Bragotto and F. G. R. Reyes, *Food Sci-Nutr.*, 5 (2017) 1039. doi: 10.1002/fsn3.499
5. A. H. Kamal, S. F. El-Malla and R. H. Elattar, *J. Fluoresc.*, 33 (2023) 1337. https://doi.org/10.1007/s10895-023-03143-0
6. M. Soyseven, H. Y. Enein and G. Arli, *Int. J. Food Sci. Technol.,* 56 (2021) 461. doi:10.1111/ijfs.14661
7. M. Soyseven and G. Arli, *J. Chromatogr. Sci.*, 60 (2022) 760. https://doi.org/10.1093/chromsci/bmab122
8. K. Kobayashi, M. Tanaka, S. Tanabe, Y. Yatsukawa, M. Tanaka and T. Suzuki, *Heliyon*, 4 (2018) e00800. doi:10.1016/j.heliyon.2018.e00800
9. M. M. S. K. Ambusaidi, S. B. S. Pandian, S. Swaminathan, and M. S. Sudhakar, *Int. J. Anal. Bioanal. Method.*, 2 (2020) 011. doi: 10.35840/2633-8912/2411
10. V. N. Krishna, D. Karthika, D. M. Surya, M. F. Rubini, M. Vishalini, Y. J. Pradeepa, *J. Young Pharm.*, 2 (2010) 297. doi: 10.4103/0975-1483.66795
11. M. Schwanninger, J. Carlos and K. Fackler, *J. Near Infrared Spectrosc.*, 19 (2011) 287. doi:10.1255/jnirs.955
12. A. Biancolillo, F. Marini, C. Ruckebusch, R. Vitale, *Appl. Sci*., 10 (2020) 1. https://doi.org/10.3390/app10186544
13. L. Wulandari, R. Idroes, T.R. Noviandy and G. Indrayanto, *Profiles of Drug Substances, Excipients, and Related Methodology Series*, (2021) 327. https://doi.org/10.1016/bs.podrm.2021.10.006
14. H. El-Mesery, H. Mao and A. Abomohra, *Sensors*, 19 (2019) 846. doi:10.3390/s19040846
15. Mujianto, Wignyanto, S. Kumalaningsih, Aulianni'am, *J. Physics: Conf. Ser.*, 1569 (2020) 032030. doi:10.1088/1742-6596/1569/3/032030
16. L. Wulandari, *Kromatografi Lapis Tipis.* Jember: P. T. Taman Kampus Presindo. Indonesia. (2011) 124. https://repository.unej.ac.id/handle/123456789/77393
17. K. T. Kyun, Statistics and Probability in *Recipes for Sciences*, (2015) Routledge, New York, 1. https://www.taylorfrancis.com/books/9781317416456/chapters/10.4324/9781315686875-6
18. L. Wulandari, K. Pratiwi and Y. Retnaningtyas, *Pak. J. Anal. Environ. Chem.*, 23 (2022) 270. doi:10.2173/pjaec/2022.12.09
19. J. S. Ribeiro, M. M. C. Ferreira and T. J. G. Salva, *Talanta,* 83 (2011) 5. doi:10.1016/j.talanta.2010.11.001
20. L. Lengkey, I. W. Budiastra, K. B. Seminar and B. S. Purwoko, *Int. J. Agric. Innov. Res.*, 2 (2013) 41. https://ijair.org/index.php/issues?view=publication&task=show&id=50
21. X. Wang, Near-infrared spectroscopy for food quality evaluation, In: Evaluation Technologies for Food Quality (J. Zhong, X. Wang, Eds.) Woodhead Publishing Series in Food Science, Technology and Nutrition (2019) 105-118. doi:10.1016/b978-0-12-814217-2.00007-x
22. H. Cheng, D. J. Garrick, R. L. Fernando, *J. Anim. Sci. Biotechnol.*, 8 (2017) 1. https://doi.org/10.1186/s40104-017-0164-6