ORIGINAL ARTICLE

The combination of Fourier-transform infrared spectroscopy with pattern recognition techniques for classification and discrimination of red snapper fish oils

Irnawati Irnawati[1], Anjar Windarsih[2], Nurrulhidayah Ahmad Fadzillah[3], Abdul Rohman[4,5], La Ode Muhammad Hazairin Nadia[6], Sofia Arlana[1], Ruslin[1]

[1]Department of Analytical Chemistry and Medical Chemistry, Study Program of Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari, [2]Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta, Indonesia, [3]International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Kuala Lumpur, Malaysia, [4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Gadjah Mada, [5]Center of Excellence, Institute for Halal Industry and Systems, Universitas Gadjah Mada, Yogyakarta, [6]Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari, Indonesia

J. Adv. Pharm. Technol. Res.

ABSTRACT

Fish oils are good sources for essential fatty acids such as omega-3 and omega-6 fatty acids needed to human growth. Indonesia is rich in fish species and among this, red snapper fish (*Lutjanus* sp.) can be extracted to get red snapper fish oils (RSFOs). The aim of this study was to classify and discriminate RSFO from different origins using Fourier-transform infrared (FTIR) spectra and pattern recognition techniques. All of the RSFO's FTIR spectra were very similar. The FTIR vibrations showed the presence of triglycerides as the main composition in fish oils. Principal component analysis (PCA) could separate the RSFO according to sample origin. Supervised pattern recognition of partial least square-discriminant analysis (PLS-DA) and sparse PLS-DA (sPLS-DA) successfully discriminated and classified different *Lutjanus* species of fish oils obtained from different origins. The vibration of functional groups at 1711, 1653, 1745, and 3012 per cm were considered for their important contributions in discriminating of *Lutjanus* species (variable importance in projection, variable importance in the projection score >1). Fish oils obtained from the same species were classified into the same class indicating similar chemical compositions. Among the three pattern recognition techniques used, sPLS-DA offers the best model for the discrimination and classification of *Lutjanus* fish oils. It can be concluded that FTIR spectroscopy in combination with the pattern recognition technique is the potential to be used for of fish oil authentication to verify the quality of the fish oils. It can be further developed as a rapid and effective method for fish oil authentication.

Key words: Fourier-transform infrared spectroscopy, *Lutjanus fish oils,* partial least square-discriminant analysis, principal component analysis, sparse partial least square-discriminant analysis

Address for correspondence:
Dr. Irnawati Irnawati,
Study Program of Pharmacy, Faculty of Pharmacy, Halu Oleo University, Kendari 93232, Indonesia.
E-mail: irnawati.vhina@gmail.com

Submitted: 16-Aug-2023 **Revised:** 16-Dec-2023
Accepted: 02-Jan-2024 **Published:** 06-May-2024

Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/JAPTR.JAPTR_401_23

INTRODUCTION

Fish is considered a sustainable source of food products needed by humans. It is well known that fish oils contain low rates of carbohydrates, saturated lipids, and cholesterol with high levels of essential fatty acids and micronutrients

How to cite this article: Irnawati I, Windarsih A, Fadzillah NA, Rohman A, Nadia LO, Arlana S, *et al.* The combination of Fourier-transform infrared spectroscopy with pattern recognition techniques for classification and discrimination of red snapper fish oils. J Adv Pharm Technol Res 2024;15:99-103.

such as polyunsaturated fatty acids including omega-fatty acids, vitamins, and minerals. Fish also contain high levels of protein needed for human development; therefore, fish can be an alternative source of protein.[1] Fish oils could be obtained by extracting oils from the corresponding oils, and among fish oils used in supplements are cod liver oil and salmon oil.[2] Fish oil supplements, particularly those with high concentrations of omega-3 fatty acids such as docosahexaenoic acids and eicosapentaenoic acid, have become more popular in recent years.[3] Omega-3 fatty acids are believed to improve human health such as for prevention of degenerative diseases of cancers, cardiovascular diseases, or Alzheimer's.[4]

Indonesia is considered a maritime country, in which two-thirds country was surrounded by oceans, as a consequence, several fish species are the potential to be cultivated as fish oils.[5] Southeast Sulawesi Province is known as the source of fishes in Indonesia, and among the most caught fishes in this province is *Lutjanus* sp. known as red snapper with the local name of *Kakap Merah*.[6] The oil content of fish is influenced by several factors including abundance of food habitat, climate, sex, age, and life stage.[7] The quality control and authenticity of fish oils are critical to guaranteeing that the products containing fish oils fit for human consumption and are not adulterated or incorrectly claimed, therefore, the determination of fish oils authenticity has been a concern among scientists. Some approaches and analytical methods have been used for quality control of fish oils and fish-based products such as proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance combined with discriminant analysis,[3,8] Raman spectroscopy,[6] metabolomics method utilizing liquid chromatography high-resolution mass spectrometry,[9] and gas chromatography hyphenated with triple quadrupole mass spectrometer.[10] These methods typically require sophisticated instruments and skillful personnel, so that simple and not complex instruments should be explored and developed for the discrimination and characterization of fish oils. One of the potential methods to be used for the discrimination of fish oils is vibrational spectroscopy,[11] mainly Fourier-transform infrared (FTIR) spectroscopy.

The responses obtained from instrumental measurement typically involved large datasets, therefore, the use of advance of multivariate data analysis or chemometrics is a must. Using chemometrics of pattern recognition techniques, the big data responses obtained from the chemical or instrumental measurements could be treated easily and could be extracted into more understandable information. The FTIR spectroscopy combined with the chemometrics technique of discriminant analysis has been successfully employed for the differentiation of fish oils including Patin fish oils (freshwater fish) typically consumed by Indonesian and other Southeast regions,[12] *Gabus* fish oil and *Bandeng* fish oil.[13] Using the literature investigation, the discrimination of fish oils of red snapper from *Lutjanus* genus is not reported, therefore, the aim of this study was to focused on the application of chemometrics of pattern recognition techniques for the discrimination of fish oils extracted from red snapper.

MATERIALS AND METHODS

Materials

Marine fishes namely *Lutjanus* genus were collected from 12 locations around the Southeast Sulawesi coast, Indonesia. All fish samples were collected fresh from free-roaming fish. The fish samples were eviscerated, filleted, and immediately placed in the cooling box, to be sent to the Laboratory of Pharmaceutical Chemistry, Haluoleo University. Upon arrival at the laboratory, the filleted fish samples were dried using an oven (Stuart Scientific) at a temperature of 70°C for 2 h × 24 h and powdered using a blender (Philips blender). The powdered sample was stored at 4°C for further analysis. The other solvents including *n*-hexane and acetone used for analysis were of pro-analytical grade and were supplied by E. Merck (Darmstadt, Germany).

Extraction of fish oils

The extraction of red snapper fish oils (RSFOs) was adapted according to the methods described by Rincón-Cervera *et al*.[14] with slight modification. Fifty grams of powdered *Lutjanus* fish were extracted with 750 mL n-hexane using a Soxhlet apparatus for 2 h at a temperature of 70°C. After the extraction was complete, a rotary evaporator (Stuart) at 50°C was used to remove the solvent. The RFSO were collected, weighed, and stored at 4°C in dark bottles for future processing.

Purification of oils

Crude RFSO was purified using bentonite at a ratio 1% (w/w) of oil weight, then centrifuged (Boeco Germany) for 5 min, filtered, and stored at dark bottle in a freezer for subsequent analysis.[15]

Fourier-transform infrared spectra measurement

The spectra acquisition was performed according to the condition as described by Irnawati *et al*.[16] An FTIR spectrophotometer (Thermo Scientific Nicolet iS10, Madison, WI) using horizontal attenuated total reflectance composed of ZnSe crystal with OMNIC software control was used to scan all oil samples. Analysis was conducted in the middle infrared region (4000–600 per cm) employing a scanning number of 32 and a resolution of 8 per cm. The background FTIR spectrum of air was used to correct all FTIR spectra of samples. This process was recorded in triplicate.

Chemometrics analysis

MetaboAnalyst 5.0 was used to carry out chemometrics techniques such as principal component analysis (PCA),

partial least square-discriminant analysis (PLS-DA), and sparse sPLS-DA (sPLS-DA). PLS-DA and sPLS-DA are supervised methods that use multivariate regression techniques to extract information through a linear combination of the original variable (x) that can predict the class members (y), while PCA is an unsupervised method that aims to determine the direction that best explains the variance in a data set without referring to class labels. The absorbance value from FTIR spectra was used as the variable in chemometrics analysis.

RESULTS AND DISCUSSION

FTIR spectra are considered fingerprinting analytical methods typically applied for the characterization and differentiation of samples such as fish oils. It means that no two fish oils have the same FTIR spectrum. The number of peaks, the exact frequencies (wavenumbers) of peaks and shoulders, and the intensities (absorbances) of peaks and shoulders could be used to distinguish edible oils and fats from each other in their FTIR spectra.[17] FTIR spectra of studied fish oils are depicted in Figure 1. It is difficult to discriminate fish oils based on their FTIR spectra since all fish oils are dominantly composed by triacylglycerols (TAGs) as represented by peaks and shoulders indicating the functional groups present in fish oils. For this reason, the employment of chemometrics of pattern recognition techniques either unsupervised or supervised is a must to make the easy understanding during the differentiation of studied fish oils.[2]

Figure 1 exhibits FTIR spectra of studied RSFO, in which each peak expressed the functional groups. To be measured using FTIR spectroscopy, the molecule must be infrared active, capable of absorbing infrared radiation to provide vibrational transitions. For example, vibrations at 2962 and 2924 per cm are representing of aliphatic CH, CH_2, and CH_3 absorption IR radiation at that region to provide the vibrational transition in the mode of asymmetrical stretching whereas vibration at 2854 per cm is associated to the stretching vibration of aliphatic CH, CH_2, and CH_3. Meanwhile, the peak at 3012 per cm is related to the stretching vibration of aromatic CH, CH_2, and CH_3. The carbonyl (C = O) absorption was demonstrated at bands of 1745 and 1711 per cm. The presence of C = C was shown at peak of 1653 per cm, meanwhile, the absorption of C-O was depicted at peak of 1200–1000 per cm. The bending vibration of CH, CH_2, and CH_3 was also found in this study which identified at peaks of 1419, 1317, 1234, 930, and 721 per cm. These results were in accordance with previous research on the use of FTIR spectroscopy to analyze fish oils.[18,19] The FTIR vibrations measured in this study were related to the vibrations of functional groups contained in fatty acids, TAGs, and other lipid compounds in RSFO.

PCA analysis on different RFSOs from different origins was first used to identify sample patterns. PCA differentiates samples by reducing the number of original variables used into several principal components (PCs) that representing the total variations.[20] In this study, PCA using the first two PCs could be used to differentiate different RSFO samples from different origins as shown in Figure 2. The first two PCs explained 65.9% and 22.4% of the variance in the data set. However, it can be seen that the separation among samples was not clear enough. Therefore, supervised pattern recognition of PLS-DA was further used to obtain better discrimination between samples. PLS-DA uses the PLS regression algorithm for discriminant analysis. The sample in PLS-DA is classified into two different classes: Class 1 which includes data from the interest class, and Class 0 which included all other classes. The class to which a sample belongs is indicated by the dependent variable vector y, which might have a value of either 0 or 1. The yi has a value of 1 if an arbitrary sample (i) is a member of the interest class; if the sample (i) is not a member of the interest class, yi has a value of 0. As a result, the approximate value of 0 and 1 represent the estimated value for yi. When samples from classes 0 and 1 have distributions of yi estimates that do not overlap, effective discriminations are produced.[21] It is significant to remember that a unique model is created for every interest class in the majority of PLS-DA applications.

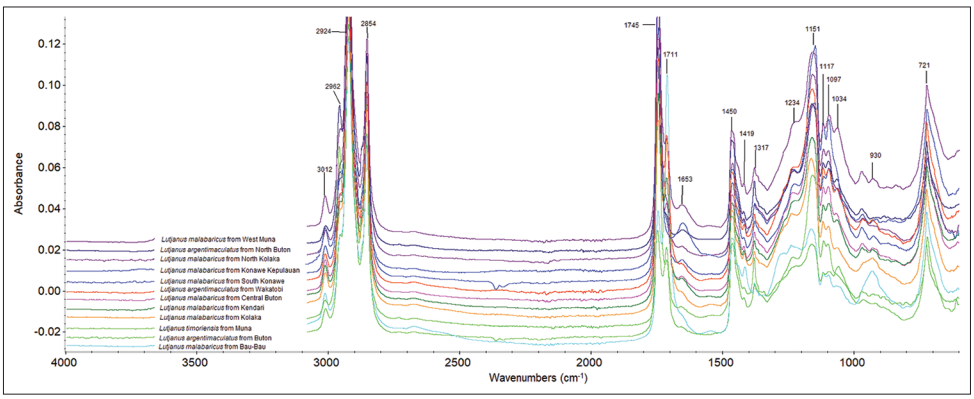

Figure 1: Fourier-transform infrared spectra of fish oils extracted from different species of *Lutjanus* fish

In this study, PLS-DA successfully discriminated RFSO from different origins as shown in Figure 3a. PLS-DA showed better classification performance compared to the PCA model. Samples of *Lutjanus* fish oils from the same species obtained from different origins were classified into the same class. It means that fish oil from the same *Lutjanus* fish species obtained from different origins have similar lipid compositions because their score plot was close to each other and classified into the same class. Through variable importance in the projection (VIP) analysis in PLS-DA models, variable of functional groups vibration which have high contributing in discriminating different *Lutjanus* species could be identified as shown in Figure 3b. Based on VIP score (>1), the vibration of functional groups at 1711, 1653, 1745, and 3012 per cm had an important role in discriminating of *Lutjanus* species. However, the PLS-DA model could only partially discriminate of *Lutjanus* species. Therefore, further analysis using sPLS-DA was performed for the classification of different RFSOs from different origins. Results showed that sPLS-DA resulted better classification than using PLS-DA. Samples of RFSO from the same species were more tightly clustered into one class as depicted in the sPLS-DA score plot in Figure 4. sPLS-DA is the extension of PLS-DA. PLS-DA and sPLS-DA differ in that sPLS-DA accomplishes the variable selection and classification processes in a single step.[22,23] Using this algorithm, sPLS-DA is capable of better discrimination and classification results. To evaluate the proceeds of PLS-DA and sPLS-DA, misclassification test was used to identify misclassified samples. Results showed that there were no misclassification findings in either the PLS-DA or sPLS-DA models, indicating to their success as models.

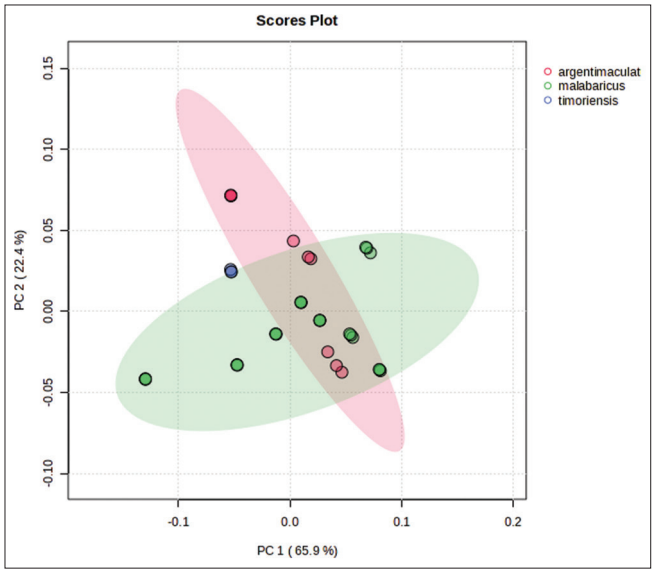

Figure 2: Principal component analysis using principal component 1 (PC1) and PC2 of different red snapper fish oils

CONCLUSION

FTIR spectroscopy could be used for fingerprinting analytical techniques for fish oils effectively, without time-consuming. Combined with pattern recognition chemometrics, either unsupervised or supervised pattern recognition, could be used for differentiation and discrimination of fish oil samples from the genus of *Lutjanus*. PCA could be used to differentiate different RFSOs from different origins. In addition, the best classification model was demonstrated by sPLS-DA, which clearly classified after PLS-DA and sPLS-DA were successfully employed to distinguish between various RSFOs from diverse origins. This finding is useful for authentication of fish oil samples for quality control purposes. Therefore, this method can be further used as a rapid and effective method for the discrimination and classification of fish oil samples using FTIR spectroscopy and pattern recognition chemometrics.

Financial support and sponsorship

This work was fully supported by Penelitian Dasar Unggulan Perguruan Tinggi grant, Ministry of Education, Culture, Research, and Technology, Republic of Indonesia, with contract number 41/UN29.20/PG/2023.

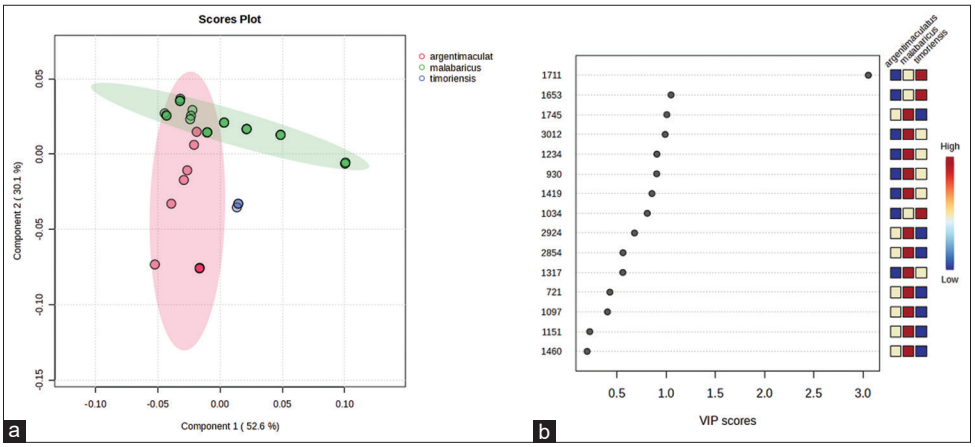

Figure 3: Two-dimensional partial least square-discriminant analysis of red snapper fish oils from different origins (a) and analysis of variable importance in the projection score (b)

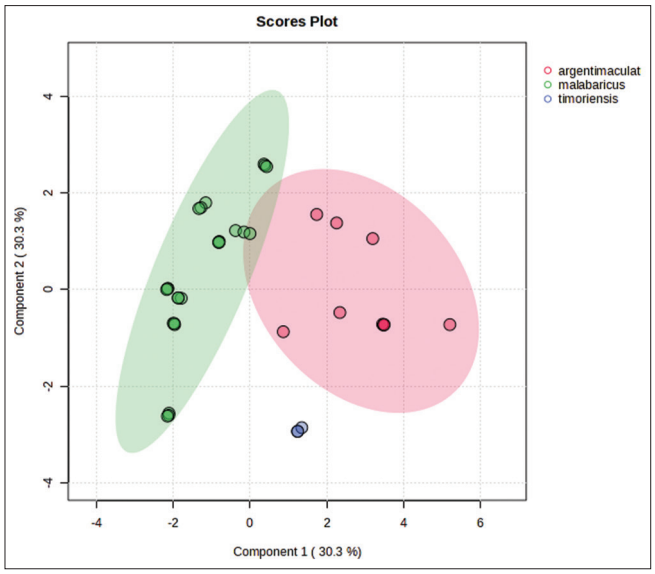

Figure 4: Two-dimensional sparse partial least square-discriminant analysis of different red snapper fish oils

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Velioğlu HM, Temiz HT, Boyaci IH. Differentiation of fresh and frozen-thawed fish samples using Raman spectroscopy coupled with chemometric analysis. Food Chem 2015;172:283-90.
2. Rohman A, Putri AR, Irnawati, Windarsih A, Nisa K, Lestari LA. The employment of analytical techniques and chemometrics for authentication of fish oils: A review. Food Control 2021;124:107864.
3. Amorim TL, Granato ÁS, de Oliveira Mendes T, de Oliveira MA, Amarante GW, de la Fuente MA, *et al.* Lipid classification of fish oil omega-3 supplements by 1H NMR and multivariate analysis. J Food Compost Anal 2021;102:104060.
4. Innes JK, Calder PC. Marine omega-3 (N-3) fatty acids for cardiovascular health: An update for 2020. Int J Mol Sci 2020;21:1362.
5. Sasongko H, Nurrochmad A, Nugroho AE, Rohman A. Indonesian freshwater fisheries' oil for health and nutrition applications: A narrative review. Food Res 2022;6:501-11.
6. Putri DN, Manshur HA, Setyawan T, Harini N. Physicochemical and fatty acid profile of fish oil from red snapper heads (*Lutjanus malabaricus*) refined from various naoh concentrations. Agrointek 2021;15:1026-37.
7. Ozogul Y, Ozogul F, Çiçek E, Polat A, Kuley E. Fat content and fatty acid compositions of 34 marine water fish species from the Mediterranean sea. Int J Food Sci Nutr 2009;60:464-75.
8. Aursand M, Standal IB, Axelson DE. High-resolution (13) C nuclear magnetic resonance spectroscopy pattern recognition of fish oil capsules. J Agric Food Chem 2007;55:38-47.
9. Windarsih A, Suratno, Warmiko HD, Indrianingsih AW, Rohman A, Ulumuddin YI. Untargeted metabolomics and proteomics approach using liquid chromatography-Orbitrap high resolution mass spectrometry to detect pork adulteration in *Pangasius* hypopthalmus meat. Food Chem 2022;386:132856.
10. Xu L, Cheng H, Müller M, Sinclair AJ, Wang W, Guo X, *et al*. Targeted quantitation of furan fatty acids in edible oils by gas chromatography/triple quadrupole tandem mass spectrometry (GC-TQ/MS). Food Chem 2023;404:134521.
11. Bekhit MY, Grung B, Mjøs SA. Determination of omega-3 fatty acids in fish oil supplements using vibrational spectroscopy and chemometric methods. Appl Spectrosc 2014;68:1190-200.
12. Putri AR, Rohman A, Riyanto S. Authentication of patin (*Pangasius micronemus*) fish oil adulterated with palm oil using Ftir spectroscopy combined with chemometrics. Int J Appl Pharm 2019;11:195-9.
13. Ikhsan AN, Rohman A, Putri AR, Syifa F, Mustafidah M, Martien R. Application of FTIR spectroscopy and chemometrics for the prediction of radical scavenging activities of fish oils. Indonesian J Pharm 2021;32:166-74.
14. Rincón-Cervera MÁ, Villarreal-Rubio MB, Valenzuela R, Valenzuela A. Comparison of fatty acid profiles of dried and raw by-products from cultured and wild fishes. Eur J Lipid Sci Technol 2017;119:201600516.
15. César QP, Alejandro AF, Lugo RD, Omar PG, Elisa JR, de la Torre J, *et al.* Assessment of new conditions for oil extraction from shark liver pool. Open Access J Sci 2020;4:17-22.
16. Irnawati, Riyanto S, Rohman A. Adulteration of gabus (*Channa striata*) fish oil with corn oil and palm oil: The use of FTIR spectra and chemometrics. Food Res 2021;5:184-90.
17. Che Man YB, Rohman A, Mansor TS. Differentiation of lard from other edible fats and oils by means of Fourier transform infrared spectroscopy and chemometrics. J Am Oil Chem Soc 2011;88:187-92.
18. Syifa F, Irnawati, Riyanta AB, Rohman A. Authentication analysis of snakehead fish oil using combination of FTIR spectra and chemometrics. Int J Pharm Res 2020;13:160-7.
19. Karunathilaka SR, Choi SH, Mossoba MM, Yakes BJ, Brückner L, Ellsworth Z, *et al.* Rapid classification and quantification of marine oil omega-3 supplements using ATR-FTIR, FT-NIR and chemometrics. J Food Compost Anal 2019;77:9-19.
20. Marikkar JM, Yanty NA. Principal component analysis of fatty acid data to detect virgin coconut oil adulteration by palm olein. CORD 2018;34:30-8.
21. Saviano G, Paris D, Melck D, Fantasma F, Motta A, Iorizzi M. Metabolite variation in three edible Italian *Allium cepa* L. by NMR-based metabolomics: A comparative study in fresh and stored bulbs. Metabolomics 2019;15:105.
22. Lê Cao KA, Boitard S, Besse P. Sparse PLS discriminant analysis: Biologically relevant feature selection and graphical displays for multiclass problems. BMC Bioinformatics 2011;12:253.
23. Jiménez-Carvelo AM, Martín-Torres S, Ortega-Gavilán F, Camacho J. PLS-DA versus sparse PLS-DA in food traceability. A case study: Authentication of avocado samples. Talanta 2021;224:121904.