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Differentiation of white rice Khao Dawk Mali 105 (KDML105) from other Thai white rice using FTIR microscope-attenuated total reflectance-focal plane array detector

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Abstract

Thai Hom Mali rice is one of Thailand's most important agricultural products, due to its higher commercial value compared to other rice, both domestically and internationally. A higher ratio of other white rice varieties is frequently found in white rice Thai Hom Mali affects consumer confidence since there is no fast procedure for differentiation of Thai Hom Mali Rice from other rice varieties, for this reason, this research proposed a spectroscopic analysis method to investigate the biochemical content of rice samples for distinguishing Thai Hom Mali Rice from other rice varieties. The absorbance spectrum of five Thai rice varieties was identified: Khao Dawk Mali 105 (KDML105), Pathum Thani 1 (PTT1), RD77, Chai Nat 1 (CN1), and Phitsanulok 2 (PLS2). The FTIR-microscope-attenuated total reflectance-focal plane array detector (FTIR-microscope-ATR-FPA detector) was applied to acquire the bandwidth of the mid-wavelength infrared (3800-900 cm^{-1}). According to the spectroscopic data, the absorbance spectrum of KDML105 rice contained a strong intensity to identify the wavenumber at 1250 and 1748 cm^{-1} representing the vibrational mode of C–N stretching in aromatic amine and C=O stretch in ester compounds, respectively. The rice varieties were classified using principal component analysis (PCA) to identify the wavenumber and separate the KDML105 rice from other rice varieties with PC1 achieving more than 82% for individual analysis and more than 40% for overall analysis, respectively.

1. Introduction

Rice (*Oryza sativa* L.) is the major energy source for people in many countries (Khush, 1997; Preeya *et al.*, 2009; Pramai and Jiamyangyuen, 2016) with Thailand being one of the world's top producers and exporters of rice, especially the Thai Hom Mali variety according to the Department of International Trade Promotion 1.15 million tons of Thai Hom Mali Rice were exported in 2021. When cooked, Thai Hom Mali rice has better aromatic, softness, and adhesive characteristics than other Thai rice varieties. With this uniqueness, as previously mentioned, Thai Hom Mali rice generates higher commercial value than other rice, both domestically and internationally. According to the Thai Agricultural Standard for Thai Hom Mali rice products, regulated by the National Bureau of Agricultural Commodity and Food Standards, all Thai Jasmine rice products must contain no less than 92% of Thai aromatic rice. However, a higher ratio of other white rice varieties is frequently found in both domestic and exported white rice Khoa Hom Mali products, regardless of unintentional production or adulteration. In fact, the current Thai Agricultural Standard clearly defines the Thai Hom Mali rice variety, under the recommended term of Thai Jasmine rice, which differs from other varieties of Thai aromatic rice according to Thai Agricultural Standard 4000-2017 (2017a) and 4001-2017 (2017b). However, the naturally strong fragrance and soft texture familiar to all consumers belong only to the Thai Hom Mali rice varieties such as Khoa Dok Mali 105 (KDML105) and RD15, and consumers often complain of less or even no aroma in Thai Jasmine rice and Thai fragrant rice or Thai aromatic rice products. This is because there are still no rapid procedures for differentiating Thai Hom Mali rice varieties KDML105 or RD15 from other varieties of Thai aromatic rice that appear to have significantly less aroma or even no unique blend of the aroma compounds present in Khoa Dok Mali 105. In fact, all the analytical methods published as testing guidelines in the Thai Agricultural

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Standard for Thai aromatic rice (TAS 4001-2017) and Thai Hom Mali (TAS 4000-2017) are only suitable for the quality classification of Thai aromatic rice products, and not for differentiating Thai Hom Mali rice from other Thai aromatic rice varieties. Furthermore, to comply with the recommended sampling procedure in these guidelines for quality control, quantitative analysis must be performed on a large number of rice samples. On the other hand, the testing procedures for identifying an admixture in Thai Jasmine rice, specifically for the Thai Hom Mali rice products, must be practical to implement in rice production plants and the certified agency.

An alternative method using Fourier transform infrared microscope-attenuated total reflectance-focal plane array detector (FTIR-microscope-ATR-FPA detector) was proposed in this paper to investigate the chemical content in the rice grains of Khoa Dok Mali 105 (KDML105) and four selected rice varieties (Pathum Thani 1 (PTT1), RD77, Chai Nat 1 (CN1) and Phitsanulok 2 (PLS2)). FTIR spectroscopy uses radiation in the mid-IR region (3800-900 cm^{-1}) to excite the vibration of the molecular bonds with the electric dipole moment and is proven to be a powerful method for investigating biological materials. In addition, when used in combination with a microscope, attenuated total reflectance, and focal plane array detector, it provides a higher spatial resolution that is closer to the diffraction limit than black body radiation sources. As a result, sensitivity and the signal-to-noise ratio are improved (Miller *et al*., 2000; Bertrand *et al*., 2011). It can be used to investigate samples in various forms such as cells and tissues. Finally, the principal component analysis technique (PCA) was used to differentiate KDML105 from other white rice varieties based on the spectroscopic data.

2. Materials and methods

2.1 Sample preparation

The rice samples used in this research were obtained from various rice research centers: Khao Dok Mali 105 rice from Ubon Ratchathani Rice Research Center (Thailand), Chai Nat 1 rice from Chainat Rice Research Center (Thailand), Phitsanulok 2 rice from Phitsanulok Rice Research Center (Thailand), and Pathum Thani 1 rice and RD77 rice from Pathum Thani Rice Research Center (Thailand). Before milling, the average moisture content of all major paddy varieties was 16% (wet basis) (measured by a moisture analyzer from Sartorius, Germany). To preserve as much of the chemical composition as possible, all rice samples were kept as paddy in a dry area. A small rice milling machine was used (Thongtawee model NW 1000 TURBO). The refinement of the milling process was based on the quality of the milled rice to ensure it had the least number of broken kernels and a similar whiteness level to commercially available rice.

Since the goal of this research was to investigate the biochemical compositions of all rice varieties, the inner surface was considered. The rice sample was attached to the sample holder with the inner surface of all grains and then exposed by slowing the removal of several layers on the outer surface (10 µm per cutting cycle) with a microtome (Figure 1). The total depth of the cutting layer was about 300 µm and the inner surface appeared totally flat.

2.2 Acquisition of the absorbance spectrum of the rice sample by an FTIR-microscope-ATR-FPA detector

The spectroscopic methods were thoroughly researched to define the identity wavenumbers of the absorbance spectrum for differentiating rice varieties. In this study, samples of Khao Dawk Mali 105 (KDML105), Pathum Thani 1 (PTT1), RD77, Chai Nat 1 (CN1), and Phitsanulok 2 (PLS2) rice varieties were used for rice classification. The FTIR-microscope-ATR-FPA detector (Bruker Tensor 27 IR and Hyperion 3000 , Bruker Optik GmbH, Ettlingen, Germany) was applied to acquire the bandwidth of the mid-wavelength infrared (3800-900 cm^{-1}) (Figure 1). The measurements were set as follows: field of view = 32.2 µm, binning = 16 × 16, scan time = 256 and wavelength resolution = 2 nm.

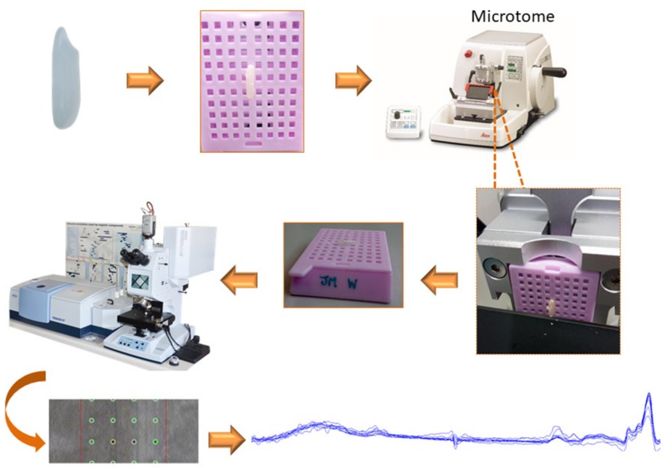

Figure 1. FTIR-microscope-ATR-FPA detector procedure for measuring rice response to IR light.

2.3 Data analysis

To define a set of wavenumbers to identify and classify white rice KDML105 from other white rice varieties, the entire spectrum data (3800-900 cm^{-1}) were baseline-corrected (rubber band correction algorithm) and normalized to reduce the effect of different radiation absorption by the vector normalizing technique. The data preprocessing of the IR spectrum was performed by the

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software OPUS 7.2. To classify white rice KDML105 from other white rice varieties by considering the identified wavenumber, the IR spectrum was smoothed using the moving average technique. Linear baseline correction (Extend Multiplicative Signal Correction (EMSC)) and vector normalization were then applied. Finally, PCA was carried out on the preprocessing spectrum using Unscrambler®X 10.5 software.

3. Results and discussion

3.1 Absorption spectrum of KDML105 rice

The average absorption spectrum of KDML105 rice at a depth of approximately 300 µm from the surface is shown in Figure 2. According to the results, the absorbance was functionally dependent on the wavenumbers which differed significantly between the sub-locations within a single 32.2 µm test point, especially when comparing the average absorbance spectrum in the transparent region with the opaque white region sub-locations. The average absorbance spectrum was higher in the transparent region than the opaque white region, showing dominant wavenumbers at 1250 and 1748 cm^{-1}. When the chemical composition of KDML105 rice was compared with the vibration type database of an infrared light source, wavenumber 1250 cm^{-1} represented the stretched vibration of the C–N aromatic amines (Mia *et al*., 2017), while wavenumber 1748 cm^{-1} represented the stretch vibration of the C=O esters (Mecozzi *et al*., 2012). According to the results, the sub-locations of the rice grains in the transparent region contained significantly different amounts of amino acids, amylose, amylopectin, lipids, volatile substances, and minerals compared to the sub-location in the opaque white region of the rice grain. Another study reported that the chalky grain region was different from the transparent region of rice grain in terms of endosperm, morphology, and chemical composition of the physical properties which may affect the eating quality (Lisle *et al*., 2000). Zhaomiao *et al*. (2016) found that in the mutant Japonica rice grain, the opaque region contained starch, a percentage of amylopectin, protein, and some amino acids, including phenylalanine, aspartate, threonine, and glutamate, all of which were lower than in mutant Japonica transparent rice grains. According to the results, the identity of KDML105 rice at wavenumbers 1250 and 1748 cm^{-1} signified the absorbance spectrum of the transparent region, but not the opaque region, as shown in Figure 2. The identity wavenumbers 1250 cm^{-1} indicates an aromatic compound. Aromatic compounds have been identified as key compounds in aromatic rice, particularly 2-acetyl-1-pyrrolin (2AP), which has been used to differentiate aromatic and nonaromatic rice (Daygon *et al.,* 2016; Sansenya *et al*., 2018; Chinachanta *et al*., 2021; Zheng *et al*., 2022) while identity wavenumbers 1728 cm^{-1} indicates lipid compound. Lipid compounds were discovered at small levels in polished rice (Kongmon *et al*., 2020). However, the amount of lipids that were low in rice could be traced using this experiment. Two wave identities were found only in transparent regions with a tight arrangement of the grain compound. The granule compounds are confirmed to be loosely arranged in regions where the white opaque grains with essential oils may be minimal or nonexistent, despite freshly processed rice attrition. The findings of this study could be used to indicate the location of the storage mechanism of the aromatic compounds in the KDML105 rice kernels. The KDML105 rice grains contain a higher amylose content as well as protein and fats, which are the source of precursors for the synthesis of aromatic compounds. Wei *et al*. (2017) examined the estimation of 2AP in different rice varieties and found that the concentration of 2AP KDML105 rice was 70 µg/kg in white rice with concentrations equal to 200 µg/kg in brown rice. A comparison of the survey results and an analysis of the identity of the absorbance spectrum of KDML105, PTT1, RD77, CN1, and PLS2 rice varieties indicate that the identity of KDML105 rice at wavenumbers 1250 and 1748 cm^{-1} differs from the four rice varieties, as reported in the next section.

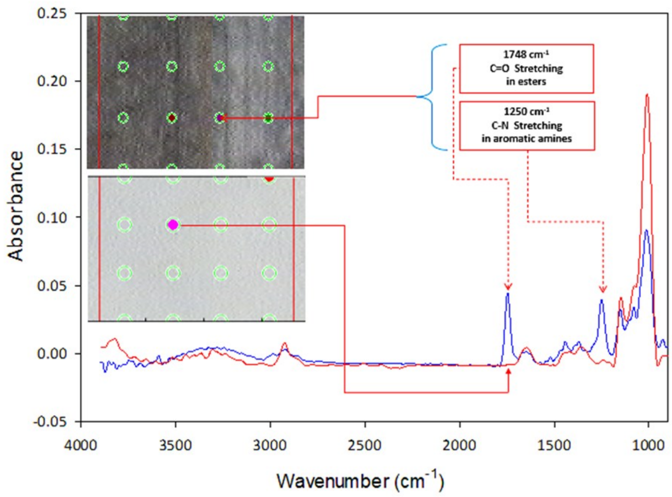

Figure 2. Comparison of the average absorbance spectrum of KDML105 rice gain between the locations in transparent and opaque white regions.

3.2 Comparison between the identity of the absorbance spectrum for KDML105, PTT1, RD77, CN1, and PLS2 rice varieties

A comparison between the average absorbance spectrum of all white rice varieties when considering the transparent region of PTT1, RD77, CN1, and PLS2 rice grains is presented in Figure 3. The spectrum of the identity wavenumber rather than the aromatic compound (2AP) at wavenumber 1250 and an ester compound of 1748 cm^{-1} did not appear to be the same as those found

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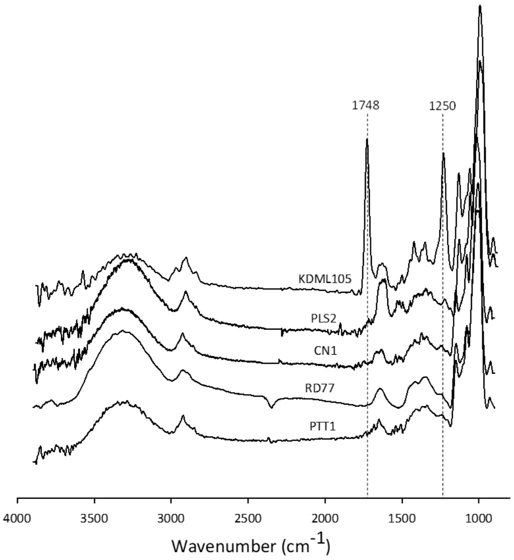

Figure 3. Comparison of the average absorption spectrum of the KDML105 rice, PTT1 rice, RD77 rice, CN1 rice and PLS2 rice varieties between the locations in the transparent region.

in the transparent region of the KDML105 grain or when comparing the absorbance spectrum of the surface within the same rice grain at the transparent and opaque white regions of the surface inside the rice grain. As can be clearly observed, the wavenumber of four white rice varieties could not be identified. Classification of other white rice varieties, from KDML105 rice by PCA analysis using wavenumbers in the range of 1800 –1200 cm^{-1}, covers the wavenumbers identified in KDML105 rice. The score plots with their corresponding loadings are shown in Figures 4 and 5. All selected white rice varieties were found to be well separated from KDML105 with PC1 achieving more than 82% for individual analysis and more than 40% for overall analysis, respectively. This research result demonstrates that this method can be used for the differentiation of KDML105 rice from other Thai white rice varieties.

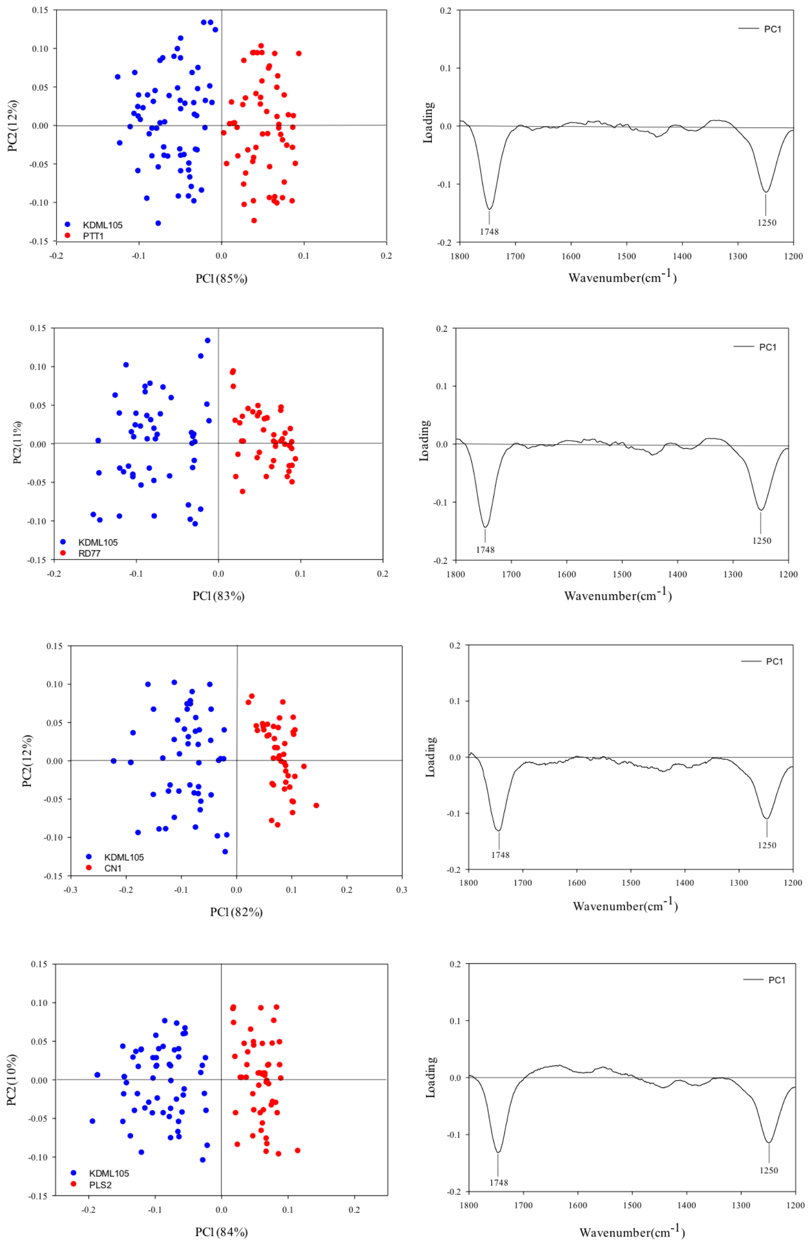

Figure 4. Score plots and their corresponding loadings in the range of 1800-1200 cm^{-1} of other Thai white rice varieties (PTT1, RD77, CN1 and PLS2) compared with the KDML105 rice (individual).

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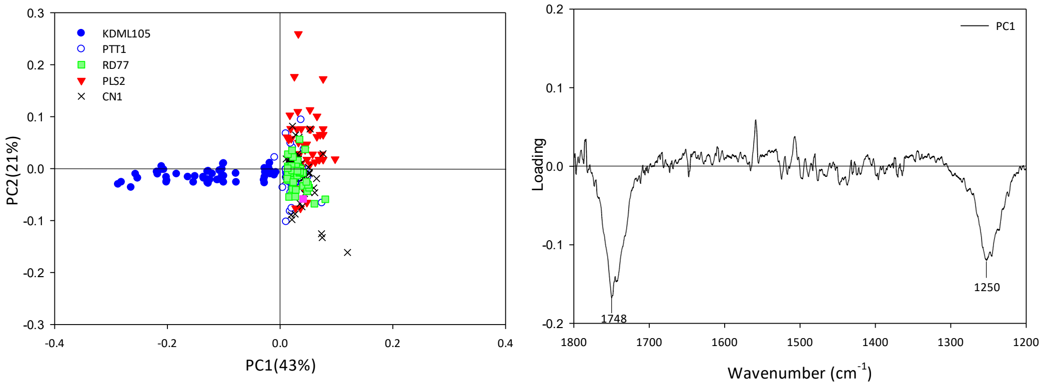

Figure 5. Score plots and their corresponding loadings in the range of 1800-1200 cm^{-1} of other Thai white rice varieties (PTT1, RD77, CN1 and PLS2) compared with the KDML105 rice (overall).

4. Conclusion

This research paper presents details of the spectroscopic analytical methods used for acquiring the identity of absorbance spectrum to differentiate the Thai white rice variety KDML105 from PTT1, RD77, CN1, and PLS2 rice varieties using an FTIR-microscope-ATR-FPA detector. The absorbance spectrum of KDML105 contained a strong intensity of the identity wavenumber at 1250 and 1748 cm^{-1} which represented the vibrational mode of C–N in aromatic amine and C=O in ester compounds, respectively. As can be clearly observed, the identity wavenumber is not found in PTT1, RD77, CN1, and PLS2 rice varieties. This conclusion is supported by the results of PCA analysis, which clearly showed that other selected Thai white rice varieties separated well from KDML105 rice.

Conflict of interest

The authors declare no conflict of interest.

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References

Bertrand, L., Robinet, L., Cohen, S.X., Sandt, C., Le Hô, A.-S., Soulier, B., Lattuati-Derieux, A. and Echard, J.-P. (2011). Identification of the finishing technique of an early eighteenth century musical instrument using FTIR spectromicroscopy. *Analytical and Bioanalytical Chemistry*, 399(9), 3025-3032. https://doi.org/10.1007/s00216-010-4288-1

Chinachanta, K., Shutsrirung, A., Herrmann, L., Lesueur, D. and Pathom-aree, W. (2021). Enhancement of the Aroma Compound 2-Acetyl-1-pyrroline in Thai Jasmine Rice (*Oryza sativa*) by Rhizobacteria under Salt Stress. *Biology*, 10(10), 1065-1080. https://doi.org/10.3390/biology10101065

Daygon, V.D., Prakash, S., Calingacion, M., Riedel, A., Ovenden, B., Snell, P., Mitchell, J. and Fitzgerald, M. (2016). Understanding the Jasmine phenotype of rice through metabolite profiling and sensory evaluation. *Metabolomics*, 12(4), 63-77. https://doi.org/10.1007/s11306-016-0989-6

Department of International Trade Promotion (2021). Fact sheet rice. Retrieved from website: https://www.ditp.go.th/ditp_web61/article_sub_view.php?filename=contents_attach/774675/774675.pdf&title=774675&cate=456&d=0

Khush, G.S. (1997). Origin, dispersal, cultivation and variation of rice. In Sasaki, T. and Moore, G. (Eds.). Oryza: From Molecule to Plant, p. 25-34. Dordrecht, Netherlands: Springer. https://doi.org/10.1007/978-94-011-5794-0_3

Kongmon, E., Jitvisate, M., Panchaisri, B., Techarang, J., Thumanu, K. and Rimjaem, S. (2020). Classification of ion-beam-induced traits in Thai jasmine rice mutants using synchrotron radiation FTIR microspectroscopy. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,* 465, 37-41. https://doi.org/10.1016/j.nimb.2019.12.029

Lisle, A.J., Martin, M. and Fitzgerald, M.A. (2000). Chalky and Translucent Rice Grains Differ in Starch Composition and Structure and Cooking Properties. *Cereal Chemistry*, 77(5), 627-632. https://doi.org/10.1094/CCHEM.2000.77.5.627

Mecozzi, M., Pietroletti, M., Scarpiniti, M., Acquistucci, R. and Conti, M.E. (2012). Monitoring of marine mucilage formation in Italian seas investigated by infrared spectroscopy and independent component analysis. *Environmental Monitoring and Assessment*, 184(10), 6025-6036. https://doi.org/10.1007/s10661-011-2400-4

https://doi.org/10.26656/fr.2017.9(1).571

Mia, S., Dijkstra, F.A. and Singh, B. (2017). Aging induced changes in biochar's functionality and adsorption behavior for phosphate and ammonium. *Environmental Science and Technology*, 51(15), 8359-8367. https://doi.org/10.1021/acs.est.7b00647

Miller, L.M., Carr, G.L., Jackson, M., Dumas, P. and Williams, G.P. (2000). The impact of infrared synchrotron radiation in biology: Past, present and future. *Synchrotron Radiation News*, 13(5), 31-38. https://doi.org/10.1080/08940880008261098

Pramai, P. and Jiamyangyuen, S. (2016). Chemometric classification of pigmented rice varieties based on antioxidative properties in relation to color. *Songklanakarin Journal of Science and Technology*, 38(5), 463-472. https://doi.org/10.14456/sjst-psu.2016.62

Preeya, P.W., Kantiwa, S., Suta, P., Paweena, P., Pichedsak, S., Pinich, W., Anan, P. and Manit, K. (2009). Cultivar and farming practice affect yield and quality of Thai rice. *Asian Journal of Food and Agro-Industry*, 2(Special Issue), 336-342.

Thai Agricultural Standard Sansenya, S., Hua, Y. and Chumanee, S. (2018). The Correlation between 2-Acetyl-1-pyrroline Content, Biological Compounds and Molecular Characterization to the Aroma Intensities of Thai Local Rice. *Journal of Oleo Science*, 67(7), 893-904. https://doi.org/10.5650/jos.ess17238

Singh, B.B., Ajeigbe, H.A., Tarawali, S.A., Fernandez-Rivera, S. and Musa Abubakar (2003). Improving the production and utilization of cowpea as food and fodder. *Field Crops Research,*84(1-2), 169-177. https://doi.org/10.1016/S0378-4290(03)00148-5

Thai Agricultural Standard. (2017a). Thai Hom Mali Rice (TAS 4000-2017). Retrieved from website: https://www.acfs.go.th/standard/download/Thai-Hom-Mali_Rice-60.pdf [In Thai].

Thai Agricultural Standard. (2017b). Thai Aromatic Rice (TAS 4001-2017). Retrieved from https://www.acfs.go.th/standard/download/Thai-Aromatic-Rice-60.pdf [In Thai].

Wei, X., Dody, D.H., Leela, P., Lisa, M. and Elmore, J.S. (2017). Evaluation of 2-acetyl-1-pyrroline in foods, with an emphasis on rice flavour. *Food Chemistry*, 232, 531-544. https://doi.org/10.1016/j.foodchem.2017.04.005

Zhaomiao, L., Deyi, Z., Xincheng, Z., Zunxin, W., Jinchao, L., Zhenghui, L., Ganghua, L., Shaohua, W. and Yanfeng, D. (2016). Chalky part differs in chemical composition from translucent part of japonica rice grains as revealed by a notched-belly mutant with white-belly. *Journal of the Science of Food and Agriculture*, 96(11), 3937-3943. https://doi.org/10.1002/jsfa.7793

Zheng, Z., Zhang, C., Liu, K. and Liu, Q. (2022). Volatile Organic Compounds, Evaluation Methods and Processing Properties for Cooked Rice Flavor. *Rice*, 15(1), 53-74. https://doi.org/10.1186/s12284-022-00602-3

https://doi.org/10.26656/fr.2017.9(1).571