Communication

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Developing a Multi-Spectral Nir Led-Based Instrument for the Detection of Pesticide Residues Containing Chlorpyrifos-Methyl in Rough, Brown, And Milled Rice

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Abstract: A recent study showed the potential of DA Perten 7200 NIR Spectrometer in detecting chlorpyrifos-methyl pesticide residue in rough, brown, and milled rice. However, this instrument is still lab-based and generally suited for point-of-sale testing in many countries. To provide a field deployable version of this technique, an existing light emitting diode (LED)-based instrument which provide discrete NIR wavelength illumination and reflectance spectra over the range of 850-1550 nm was tested. Spectra were collected from rough, brown, and milled rice at different pesticide concentrations and analysed for quantitative and qualitative measurement using partial least squares regression (PLS) and discriminant analysis (DA). Simulations for LED-based instruments were also evaluated using segments of spectra from the DA7200 to represent LED illumination. For the simulation of the existing LED-based instrument (LEDPrototype1) using their wavelengths range yielded 70.4% to 100% correct classification. Simulation of a second LED instrument, LEDPrototype2, with spectral segments selected based on significant wavelength regions from PLS regressions coefficients obtained from the DA7200 showed improved performance with R^2 of 0.59 to 0.82 and correct classifications of 71.3 to 100%. An actual LED based instrument with this capability could provide a quick screening tool to determine if MRLs are exceeded.

Keywords: chlorpyrifos-methyl pesticide residue (CMPR); detection; LED; NIR; organophosphates; pesticide residue; rice

1. Introduction

To ensure proper handling and safe consumption of rice, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have imposed maximum residue limits (MRLs) for toxic pesticides [1], including chlorpyrifos-methyl. This pesticide has been reported to have toxic effects on the human body, particularly on the brain and nervous system, if used inappropriately and excessively [2,3]. Based on independent epidemiological, in vivo, and in vitro studies, the evidence points to adverse health effects from exposure to chlorpyrifos on the developing nervous system, associated with lowered IQ at school age [3].

Rice is the main staple food in the Philippines and to ensure ample supply, importation from countries such as Thailand and Vietnam has been a common practice. In 2018, a shipment of 330,000 bags of 50 lb milled rice from these two countries was found to be infested by rice weevils and were then treated with formalin-laced insecticide by the National Food Authority (NFA) [4]. This highlighted the need to detect pesticide residues as the consumers expressed concern for the potential risk to human health, especially when pesticide residues remain undetermined during handling and before human consumption. However, the commonly used detection technique for the presence of pesticide residues in grains has been gas and liquid chromatography, which involves specialized instrumentation and labor-intensive protocol for extraction, centrifugation, clean up, evaporation, and scan of rice samples. The development of a technique or simple low-cost instrument with a fast turnaround in the detection of pesticide residues will highly benefit consumers, safety regulators, and the rice industry.

Near-infrared spectroscopy (NIRS) is a rapid, precise, and non-destructive technique that has shown potential in the determination of numerous chemical and physical properties of foods and food products. An earlier study conducted by Rodriguez et al. [5] showed the potential of using NIRS for detecting pesticide residues that contain varying concentrations of chlorpyrifos-methyl in rough, brown, and milled rice using a commercial full wavelength (950-1650 nm) NIR instrument (Perten DA7200, Perten Industries, Springfield, IL). Chlorpyrifos-methyl pesticide was selected as the target pesticide residue for investigation in this study because of the widespread use of chlorpyrifos for control of insect infestation in the Philippines and the underlying health concerns related to this pesticide. While the NIR spectroscopy technique is simple and the commercially available instrument can be readily adapted with the development of calibrations, there is a need in countries such as the Philippines, for a fast and reliable technique or instrument that is low-cost and made of locally available parts and which is preferably portable to allow testing at various handling and pre-consumption points.

An existing tabletop LED-based prototype instrument being developed at the U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Center for Grain and Animal Health Research in Manhattan, Kansas based on the design from the USDA-ARS multi-spectral high-speed sorter [6] provided a potential platform for an instrument to be used for detection of pesticide residues. The prototype, to be referred to as LEDPrototype1, was designed for bulk samples and uses an array of NIR LEDs, an NIR detector, and a microprocessor. Although the wavelengths of the LEDs in the LEDPrototype1 were not the same combination as what was found to be important in earlier studies by Rodriguez et al. (2020) using a commercial spectrometer, the evaluation of the prototype was considered important to determine potential contributions from other wavelengths and also to verify the light, signal, and noise filtering capabilities of the LEDPrototype1.

The main objectives of this study were to (1) simulate the performance of a proposed multi-spectral NIR LED-based instrument that uses the wavelengths that were identified to be important for the detection of varying concentrations of chlorpyrifos-methyl residues in various rice types (rough, brown, and milled rice), which will be based on Perten DA7200 spectral data for the identified wavelengths (to be referred to as DA7200 analog) and (2) evaluate the existing LEDPrototype1 to obtain relevant design considerations such as light, signal, and noise filtering capabilities. Findings from this study will be used for future work on the design and development of an LED-based NIR instrument that can be fabricated using low-cost and available parts in the Philippines. Considering the versatility of NIR spectroscopy at measuring and detecting different chemical compositions, it is also possible that the instrument being developed can be used for other applications such as the measurement of moisture, protein, oil, and starch contents for different grain and food products.

2. Materials and Methods

2.1. Rice Grain Samples

The samples of rough, brown, and milled rice used in this study were the same samples used by Rodriguez et al. (2020). Briefly, five pesticide-free rough rice varieties (CL151, Diamond, Hybrid 1, Gemini, and Hybrid 2) were provided to USDA-ARS Manhattan KS by the Delta Water Management Research Unit of USDA-ARS, Harrisburg, Arkansas. All rough rice samples were cleaned using the

Carter Day dockage tester (Carter Day International, Minneapolis, Minnesota, USA) and conditioned to 14% using an environmental chamber (Percival Intellus Control System, Percival Scientific, Fontana, Wisconsin, USA). The chamber was set at 23^{o}C and 73% relative humidity and samples spread on trays were conditioned inside for three days. Brown rice samples were prepared by hulling using the JLGL-45 rubber roller (Wuhan Acme Agro-Tech Co. Ltd., Wuhan, China). A portion of the brown rice was then further polished using a Twinbird MR-E500 mill (Twinbird Corp., China) to obtain milled samples. The samples were divided into 110 g subsamples using a Boerner divider (Sedburo Equipment, Des Plaines, Illinois, USA) and were placed and sealed in 946 ml clear, wide-mouth plastic jars.

For the application of treatment in rice samples, each of the 90 sub-samples for each rice type was spread onto a layer of kraft paper and sprayed evenly with 0.2 mL of water for the control samples (no pesticide applied) and 0.2 mL of pesticide concentrations for the rest of the samples that were treated with varying levels of pesticide solution containing chlorpyrifos-methyl. The pesticide solution was prepared by diluting StorcideTM II (21.60 % chlorpyrifos-methyl) with water to attain target pesticide concentrations, i.e., (a) 1.5, 3, 6, 9 and 12 ppm for rough rice, (b) 0.75, 1.5, 3, 4.5 and 6 ppm for brown rice, and (c) 0.1, 0.2, 0.4, 0.6 and 0.8 ppm for milled rice samples. The treated rough and milled rice samples were immediately placed back in the plastic jars and sealed before spectral collection. The treated brown rice samples were vacuum sealed using a FoodSaver Vac 360 (Sunbeam Products, Inc., Boca Raton, Florida, USA) to minimize lipid degradation during storage, before spectral data collection.

2.2. Instrumentation

Two NIR instruments were evaluated for their potential to detect chlorpyrifos-methyl pesticide residues, CMPR, of varying concentrations in rice (rough, brown, and milled). The instruments were a commercially available Perten DA7200 (Perten Industries, Springfield, Illinois, USA), and an existing LED-based NIR prototype instrument developed at the ARS-USDA, Center for Grain and Animal Health Research, Manhattan, KS, referred to as LEDPrototype1. Both are designed for bulk analysis.

The DA7200 spectrum ranges from 950 to 1650 nm and uses a 256-element Indium-Gallium Arsenide (InGaAs) diode detector array which is thermoelectrically cooled. The instrument collects spectral data on samples placed in an open-faced sampling dish in ambient room light at ~15 spectra/s.

The LEDPrototype1 instrument, Figure 1, was based on a design used for a multi-spectral high-speed, single seed sorter [6]. The LEDPrototype1 instrument circuit board is composed of LED light sources, an InGaAs detector, a signal amplifier, and a microprocessor. The LEDs in this instrument were selected to cover a broad spectrum range from LED wavelengths that were commercially available. LED wavelengths were 850, 910, 940, 970, 1070, 1200, 1300, 1450 and 1550 nm. LEDs were arranged in a circular pattern around the lens and directed to a central point on the grain surface 12 cm directly above the lens. The LEDs emitted a narrow light beam that was approximately dispersed over ±10^{o} from the center, Figure 2. Data acquisition and LED sequential pulsing were achieved using a microcontroller (ATmega328P Atmel Corp., San Jose, CA, USA). An InGaAs photodiode (SD060-11-41-211, Luna Optoelectronics, Camarillo CA, USA) with high sensitivity, low noise, and 1mm diameter active area for spectral detection (800-1700 nm) was used to detect reflected light and was amplified by a trans-impedance amplifier (OPA2380, Texas Instruments, Dallas TX, USA). The sensor board was placed inside a black enclosure to eliminate ambient light; communication to the laptop for data collection was via USB. A graphical user interface program (GUI) was created such that the instrument can connect to a laptop COM port at 115200 baud rate and control some of the acquisition parameters. The Atmel AVR Studio 5.1 (Atmel Corp., San Jose, CA, USA) was used to program the microcontroller to send and receive digital and analog I/O and download data to the laptop. Spectral collection on 100 g samples was done by rotating a shallow circular dish containing the sample, 76 mm diameter x 38 mm deep, placed on a small rotary table turning at 6 rpm. The sensor circuit board faces downward toward the sample being scanned.

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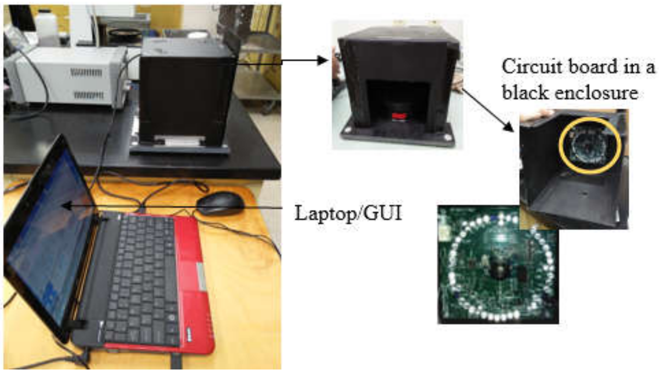

Figure 1. Photo of the vis/NIR LED-based instrument Prototype1 showing.

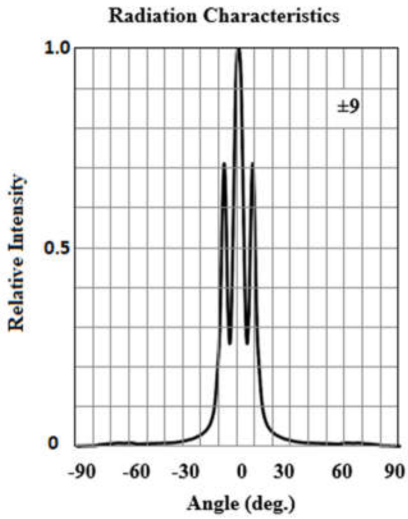

Figure 2. Angular emission characteristics for LEDs, L1300-6 shown. *(Source: Marubeni America Corp, n.d.).*

2.3. Spectral Data Acquisition

The two instruments were used to obtain spectra for each of the 90 rice samples. For the DA7200 and LEDPrototype1, three replicates were done for each sample with three repacks per replicate. The bulk rice sample to be scanned was placed in the sample dish and set in the viewing area of each of the instrument. To address potential issues of cross-contamination of pesticide residues, the sample dishes used for both instruments were washed with soap and water and then immediately dried using compressed air after spectral collection.

2.4. Data Analysis

Quantitative and qualitative spectral data analyses were performed using partial least squares (PLS) regression and discriminant analysis (DA), respectively, using the UnscramblerX version 10.5.1 (CAMO Software, Oslo, Norway). For both analyses, the 270 spectral data for each rice type were divided into a calibration (n=216) and independent validation (n=54) sample sets based on the leave-one-variety-out sampling method. This method involved using the spectral data for different pesticide residue concentrations from four of the five varieties as calibration samples while the spectra from the remaining one rice variety were used as the independent validation samples. Likewise, an analysis that used all varieties with cross validation was also performed but with no independent validation. Several pre-treatments including mean-centering, standard normal variate, multiplicative scatter correction, and derivative techniques were evaluated. The performance of the models when using the different pre-treatments were similar, as also observed by Rodriguez et al. (2020), thus only the simplest models that made use of mean-centering were presented.

For quantitative analysis, the pesticide concentrations for the sample treatments were used as reference data, i.e., 1.5, 3, 6, 9 and 12 ppm for rough rice, 0.75, 1.5, 3, 4.5 and 6 ppm for brown rice, and 0.1, 0.2, 0.4, 0.6 and 0.8 ppm for milled rice samples. For qualitative analysis, the six levels of pesticide concentration were pooled into two groups: low pesticide level (LPL) and contaminated or high pesticide level (HPL). For rough rice, the LPL group included 3 ppm and below while the HPL was 6 ppm and higher. For brown rice, the LPL group included 1.5 ppm and below and for HPL, 3 ppm and higher. Milled rice LPL was 0.2 and below and HPL was 0.4 ppm and higher. A value of "1" was assigned to LPL and "2" for HPL samples for discriminant analysis.

3. Results

The wavelengths that contributed most towards the quantitative and qualitative measurements of pesticide residues containing varying concentrations of chlorpyrifos-methyl in rough, brown, and milled rice based on earlier evaluations using DA7200 were provided by Rodriguez et al. (2020), as summarized in Table 1. These were identified based on the peaks and valleys of the plotted regression coefficients for the full wavelength (950 to 1650 nm), which represent the wavelengths that contributed most to the prediction capability of the model. Based on the functional groups present in chlorpyrifos-methyl, potential changes in the composition of the grains, and their respective adsorption wavelengths [7,8], the significant wavelengths identified for rough rice were; 980 nm (starch), 1050, 1390, and 1410 nm (oil), 1200 nm (C-H bonds), 1360 nm (methyl), 1425 nm (protein), 1480 nm (N-H bonds), and 1540 nm (amine). Important wavelengths for brown rice were 1200 and 1300 nm which (C-H bonds), 1360 nm (methyl), 1450 nm (C=O bonds), 1470 nm (N-H bonds), and 1580 nm (amide); while those for milled rice included 1200 nm (C-H bonds), 1360 nm (methyl), 1410 nm (O-H bonds), 1510 nm (oil), and 1540 nm (amine). Of these wavelengths, three were already being utilized in the LEDPrototype1, i.e., 1200, 1300, and 1450 nm. Other LEDs available in the LEDPrototype1 included 850, 910, 940, 1070, and 1550 nm.

Table 1. Summary of significant wavelengths based on calibration models developed for rough, brown, and milled rice using the Perten DA7200 and the list of discrete wavelengths fitted in the USDA-ARS vis/NIR LED-based prototype1 and recommendations for NIR LED-based prototype2.

LED Wavelength, nm	[a]Marubeni LED	DA7200 significant prediction wavelengths			USDA-ARS NIR LED-based Prototype1 (LEDPrototype1)	Proposed USDA-ARS NIR LED-based Prototype2 (LEDPrototype2)
		Rough Rice	Brown Rice	Milled Rice		
850					√	
910					√	
940					√	
970					√	
980	L980-06					√√
1050		X				√√
1070					√	
1200	L1200-06	X	X	X	√	√√
1300	L1300-06		X		√	√√
1360		X	X	X		
1390		X				

1410		X		X		
1425		X				
1450	L1450-06		X		√	√√
1470			X			
1480		X				
1510				X		
1540		X		X		
1550	L1550-06				√	√√
1580			X			
1600	L1600-06					√√
1650	L1650-06					√√

X = important wavelengths based on calibrations. √ = LED wavelengths used in existing vis/NIR LED-based instrument. √√ = recommended wavelengths for proposed LED-based Protoype2 based on performance comparisons between Perten DA7200 and LED-based Prototype1 and LED availability [a] Marubeni America Corp., Santa Clara, CA, USA [9].

An instrument simulation, referred to as the DA7200 analogue, used wavelengths within 80% relative radiant intensity of the center wavelength used in LEDPrototype1 but using DA7200 spectra for developing calibration models from these discrete ranges. Ranges from DA7200 spectra included 1055 to 1085 nm, 1185 to 1215 nm, 1275 to 1320 nm, 1420 to 1470 nm, and 1515 to 1580 nm. The prediction statistics for quantitative analysis, Table 2, shows that independent validation for rough rice had R^2s of 0.52 to 0.71 and SEPs of 2.28 to 2.95; for brown rice, R^2s of 0.58 to 0.70 and SEPs of 1.17 to 1.38 were observed, while milled rice models had R^2s of 0.57 to 0.71 and SEPs of 0.16 to 0.19. These results indicated poor to marginal quantitative prediction of CMPR at best. Qualitative predictions at different thresholds based on rice milling treatment yielded the highest percent correct classification (%CC) for milled rice at 90.7 to 100 %CC followed by rough rice, 77.8 to 92.6 %CC, then brown rice at 70.4 to 88.6 %CC, Table 3. These provided indications that using the limited wavelength regions in the LEDProtoype1 should provide good qualitative predictions of CMPR in milled rice.

Table 2. PLS model prediction statistics for determination of chlorpyrifos-methyl residues based on the DA7200 analogue for available wavelengths[a] in the LEDPrototype1 instrument.

Model Data	Calibration						Independent Validation		
	N	F	R^2 Cal	RMSEC	R^2 CV	SECV	N	R^2	SEP
Rough Rice (0 to 12 ppm)									
ALL varieties	270	8	0.68	2.39	0.60	2.66	-	-	-
CL151	216	8	0.70	2.31	0.63	2.58	54	0.56	2.91
Diamond	216	8	0.70	2.32	0.62	2.62	54	0.52	2.95
Hybrid1	216	8	0.66	2.47	0.57	2.79	54	0.71	2.28
Gemini	216	8	0.67	2.41	0.58	2.74	54	0.63	2.66
Hybrid2	216	8	0.69	2.35	0.60	2.68	54	0.62	2.65
Brown Rice (0 to 6 ppm)									

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ALL varieties	269	7	0.66	1.24	0.63	1.30	-	-	-
CL151	216	7	0.67	1.22	0.63	1.29	53	0.63	1.31
Diamond	215	7	0.66	1.23	0.63	1.30	54	0.63	1.29
Hybrid1	215	7	0.67	1.22	0.64	1.28	54	0.58	1.38
Gemini	215	7	0.66	1.23	0.62	1.30	54	0.67	1.22
Hybrid2	215	7	0.65	1.26	0.61	1.33	54	0.70	1.17
Milled Rice (0 to 0.8 ppm)									
ALL varieties	270	8	0.73	0.15	0.69	0.16	-	-	-
CL151	216	8	0.76	0.14	0.71	0.15	54	0.58	0.19
Diamond	216	8	0.74	0.15	0.69	0.16	54	0.67	0.16
Hybrid1	216	8	0.74	0.15	0.69	0.16	54	0.68	0.16
Gemini	216	7	0.69	0.16	0.65	0.17	54	0.71	0.16
Hybrid2	216	6	0.68	0.16	0.64	0.17	54	0.70	0.16

[a] Wavelengths at 80% relative radiant intensity 1055-1085 nm, 1185-1215 nm, 1275-1320 nm, 1420-1470 nm and 1515-1580 nm. N=number of samples; F=PLS factor number; R^2 coefficient of determination; RMSEC=root mean square error of calibration; Cal = calibration; CV = cross validation; SECV Standard error cross validation; SEP=standard error of prediction.

Actual instrument tests were conducted to detect CMPR using the existing design configuration of the LEDPrototype1. Table 4 summarizes the PLS model calibration and validation statistics for quantitative determination in rough, brown, and milled rice. Validation results were poor to very poor in all cases. Across the five calibration models, independent validation results for rough rice had R^2s of 0.23 to 0.59 while brown and milled rice R^2s were less than 0.03. Discriminant analysis showed the %CC for the five one-variety-out calibration models showed some potential for rough rice yielding 71.6 to 85.8 %CC. Brown rice and milled rice were poorer with %CC ranging from 58.0 to 63.6 and 55.6 to 61.1, respectively, Table 5.

Table 3. Discriminant model prediction statistics for determination of chlorpyrifos-methyl residues based on the DA7200 analogue for available wavelengths[a] in the LEDPrototype1 instrument.

Model Data	Calibration			Independent Validation		
	False Positive	False Negative	Overall % CC	False Positive	False Negative	Overall % CC
Rough Rice (Low: ≤ 3.0 ppm, High: >3 ppm)						
ALL varieties	9/135	12/135	84.4	-	-	-
CL151	7/108	7/108	87.0	5/27	4/27	83.3
Diamond	6/108	8/108	87.0	7/27	5/27	77.8
Hybrid1	4/108	6/108	90.7	2/27	8/27	81.5
Gemini	7/108	10/108	84.3	4/27	1/27	90.7
Hybrid2	10/108	7/108	84.3	4/27	0/27	92.6
Brown Rice (Low: ≤ 1.5 ppm, High: >1.5 ppm)						

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ALL varieties	30/134	7/135	72.6	-	-	-
CL151	17/108	6/108	78.7	4/26	2/27	88.6
Diamond	20/107	6/108	75.9	7/27	4/27	79.6
Hybrid1	24/107	6/108	72.2	9/27	1/27	81.5
Gemini	23/107	6/108	73.1	3/27	13/27	70.4
Hybrid2	20/107	5/103	76.9	9/27	1/27	81.5
Milled Rice (Low: ≤ 0.2 ppm, High: >0.2 ppm)						
ALL varieties	0/135	0/135	100.0	-	-	-
CL151	0/108	0/108	100.0	0/27	0/27	100.0
Diamond	0/108	0/108	100.0	5/27	0/27	90.7
Hybrid1	0/108	0/108	100.0	0/27	0/27	100.0
Gemini	0/108	0/108	100.0	0/27	0/27	100.0
Hybrid2	0/108	0/108	100.0	0/27	0/27	100.0

[a] Wavelengths at 80% relative radiant intensity for Prototype1 includes 1055-1085 nm, 1185-1215 nm, 1275-1320 nm, 1420-1470 nm, and 1515-1580 nm. % CC=percent correct classification.

There was a substantial reduction in prediction performance between the LEDPrototype1 DA7200 analog compared to actual sample tests using the LEDPrototype1 instrument. For example, milled rice that can potentially be discriminated as containing low versus high CMPR with 90.7 to 100 %CC, was only at 55.6 to 61.1 %CC in the actual test. This may indicate that while the wavelengths that are needed for effective prediction are available, the capability was not fully utilized which may highlight the need for improving the instrument design with a focus on improving light signal and noise filtering.

Based on an evaluation of the results from PLS analysis for the DA7200 and LEDPrototype1 and working within currently available LEDs, the following LEDs were selected for use in the proposed NIRLEDPrototye2, wavelengths 980, 1050, 1200, 1300, 1450, 1550, 1600, and 1650 nm. NIR region wavelengths of 1360, 1390, 1410, 1425, 1470, 1480, 1510, 1540, and 1580 nm were considered important for residue detection but are not commercially available although there is some overlap for those used in some cases.

Table 4. PLS model prediction statistics for determination of chlorpyrifos-methyl residues using the actual LEDPrototype1 instrument.

	Calibration						Independent Validation		
Model Data	N	F	R^2 Cal	RMSEC	R^2 CV	SECV	N	R^2	SEP
Rough Rice (0 to 12 ppm)									
ALL varieties[a]	810	4	0.43	3.20	0.42	3.23	-	-	-
CL151[b]	648	4	0.39	3.30	0.38	3.33	162	0.59	2.75
Diamond[b]	648	5	0.42	3.23	0.40	3.27	162	0.51	2.98
Hybrid1[b]	648	5	0.41	3.23	0.40	3.28	162	0.53	2.92
Gemini[b]	648	5	0.50	2.98	0.49	3.03	162	0.23	3.87
Hybrid2[b]	648	4	0.47	3.06	0.46	3.10	162	0.24	3.71
Brown Rice (0 to 6 ppm)									

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ALL varieties[a]	810	1	0.01	2.10	0.01	2.11	-	-	-
CL151[b]	648	5	0.10	2.00	0.07	2.04	162	0.03	2.10
Diamond[b]	648	5	0.10	2.00	0.07	2.04	162	0.01	2.13
Hybrid1[b]	648	6	0.10	2.00	0.07	2.04	162	0.01	2.13
Gemini[b]	648	1	0.01	2.10	0.00	2.11	162	0.03	2.11
Hybrid2[b]	648	1	0.01	2.10	0.01	2.11	162	0.00	2.11
Milled Rice (0 to 0.8 ppm)									
ALL varieties[a]	810	3	0.05	0.27	0.04	0.28	-	-	-
CL151[b]	648	3	0.04	0.28	0.03	0.28	162	0.01	0.28
Diamond[b]	648	3	0.06	0.27	0.04	0.28	162	0.01	0.29
Hybrid1[b]	648	3	0.08	0.27	0.07	0.27	162	0.01	0.29
Gemini[b]	648	3	0.08	0.27	0.06	0.27	162	0.01	0.28
Hybrid2[b]	648	1	0.00	0.28	0.00	0.28	162	0.02	0.28

[a] Cross validation model; [b] Independent validation set; N=number of samples; F=PLS factor number; R^2 coefficient of determination; RMSEC=root mean square error of calibration; Cal = calibration; CV = cross validation; SECV Standard error cross validation; SEP=standard error of prediction.

As noted earlier, these wavelengths are not discrete, and absorption bands for water, protein, starch, cellulose, and oil/fat can occur over a range of several nanometers [10]. For example, the protein absorption band at 1186 nm and starch band at 1200 nm can potentially be detected using an LED that covers both of these wavelength ranges. Figure 3 provides an overlapped representation of the relative spectral emission for the LEDs selected for the proposed LEDPrototype2 as provided by the manufacturer, Marubeni America Corporation, Santa Clara, CA, USA. As an example, the 1540 nm wavelength was among the identified important model wavelengths and pertains to amine absorption (Workman and Weyer, 2008); amine is a functional group present in chlorpyrifos-methyl. The 1540 nm LED is not commercially available, thus, the 1550 nm LED (1500 to 1600 nm full range; 1515 to 1580 nm at 80% relative radiant intensity) was selected as a substitute. The 1360 nm wavelength which is relevant to methyl absorption, is likewise a functional group present in chlorpyrifos-methyl but was not represented in the proposed LEDPrototype2 instrument because the closest available LED was 1300 nm (1250 to 1350 nm at full range; 1275 to 1320 nm at 80% relative radiant intensity). As more LED wavelengths become available, important wavelength(s) for specific applications can be added to potentially improve performance and also widen the range of applications. For the LEDPrototype2, considering that the emission spectra of the LEDs approximated a Gaussian curve with a full width at half maximum of approximately 50 nm centered about the peak emission wavelength [6], replacement LED wavelengths selected accounted for most of the wavelengths that were considered important for detection of CMPR.

Table 5. PLS model prediction statistics for determination of chlorpyrifos-methyl residues using the actual LEDPrototype1 instrument.

Model Data	Calibration			Independent Validation		
	False Positive	False Negative	Overall % CC	False Positive	False Negative	Overall % CC
Rough Rice (Low: ≤ 3.0 ppm, High: ≥6.0 ppm)						

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ALL varieties[a]	126/405	52/405	56.0	-	-	-
CL151[b]	107/324	44/324	53.4	21/81	2/81	85.8
Diamond[b]	102/324	42/324	55.6	20/81	8/81	82.7
Hybrid1[b]	99/324	41/324	56.8	15/81	17/81	80.3
Gemini[b]	87/324	37/324	61.7	27/81	19/81	71.6
Hybrid2[b]	100/324	27/324	60.8	36/81	9/81	72.2
Brown Rice (Low: ≤ 1.5 ppm, High: ≥3.0 ppm)						
ALL varieties[a]	153/405	163/405	22.0	-	-	-
CL151[b]	126/324	137/324	18.8	6/81	55/81	62.4
Diamond[b]	116/324	138/324	21.6	34/81	25/81	63.6
Hybrid1[b]	111/324	138/324	23.1	57/81	5/81	61.7
Gemini[b]	118/324	133/324	22.5	4/81	62/81	59.3
Hybrid2[b]	133/324	136/324	17.0	32/81	36/81	58.0
Milled Rice (Low: ≤ 0.2 ppm, High: ≥0.4 ppm)						
ALL varieties[a]	120/405	143/405	35.1	-	-	-
CL151[b]	139/324	144/324	12.7	62/81	1/81	61.1
Diamond[b]	120/324	145/324	18.2	12/81	58/81	56.8
Hybrid1[b]	112/324	140/324	22.2	34/81	33/81	58.6
Gemini[b]	101/324	119/324	32.1	24/81	47/81	56.2
Hybrid2[b]	119/324	129/324	23.5	32/81	40/81	55.6

[a] Cross validation model, [b] Independent validation set. % CC=percent correct classification.

A simulation (DA7200 analogue) was carried out to obtain an indication of the performance of the proposed LEDPrototype2. Most of the wavelengths available in the 950 to 1650 nm DA7200 spectrum, are covered in the selected LEDs for NIRLEDPrototype2 at 80% relative radiant intensity and as such, performances of the two instruments were found to be comparable, Figure 2. The wavelengths used pertain to amines, oil, protein, water, and starch [7,8]. Tables 6 and 7 provide the PLS analysis (quantitative) and discriminant analysis (qualitative), respectively, for the proposed LEDPrototype2.

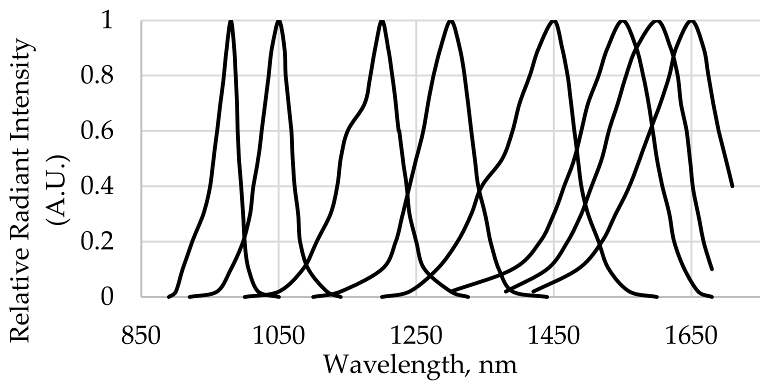

Figure 3. Plots showing overlapped relative spectral emissions of LEDs for wavelengths that were selected for the proposed NIR LED-based Prototype2 instrument *(Source: Marubeni America Corp, n.d.).*

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Table 6. PLS model prediction statistics for determination of chlorpyrifos-methyl residues using the DA7200 analogue for selected wavelengths[a] of the NIRLED-based Prototype 2 instrument.

Model Data	Calibration						Independent Validation		
	N	F	R^2 Cal	RMSEC	R^2 CV	SECV	N	R^2	SEP
Rough Rice (0 to 12 ppm)									
ALL RR varieties	270	109	0.74	2.2	0.63	2.6	-	-	-
CL151	216	10	0.72	2.2	0.64	2.5	54	0.59	2.85
Diamond	216	1010	0.75	2.1	0.64	2.5	54	0.63	2.62
Hybrid1	216	10	0.73	2.2	0.63	2.6	54	0.74	2.24
Gemini	216		0.76	2.1	0.65	2.5	54	0.64	2.71
Hybrid2	216		0.76	2.1	0.66	2.5	54	0.61	2.70
Brown Rice (0 to 6 ppm)									
ALL BR varieties	269	8	0.78	0.99	0.75	1.07	-	-	-
CL151	216	8	0.78	0.99	0.74	1.07	53	0.74	1.12
Diamond	215	8	0.77	1.01	0.74	1.08	54	0.77	1.03
Hybrid1	215	8	0.77	1.01	0.74	1.08	54	0.78	1.02
Gemini	215	8	0.79	0.98	0.76	1.05	54	0.75	1.07
Hybrid2	215	8	0.78	1.00	0.74	1.08	54	0.78	1.02
Milled Rice (0 to 0.8 ppm)									
ALL MR varieties	270	8	0.76	0.14	0.73	0.15	-	-	-
CL151	216	8	0.78	0.13	0.74	0.14	54	0.67	0.17
Diamond	216	8	0.76	0.14	0.72	0.15	54	0.75	0.14
Hybrid1	216	8	0.78	0.13	0.73	0.15	54	0.74	0.14
Gemini	216	8	0.76	0.14	0.71	0.15	54	0.82	0.13
Hybrid2	216	8	0.78	0.13	0.73	0.15	54	0.71	0.16

[a] Wavelengths at 80% relative radiant intensity for Prototype 2 includes 970-985 nm, 1035-1060 nm, 1185-1215 nm, 1275-1320, 1420-1470 nm, 1515-1580 nm, 1555-1630, and 1620-1680 nm; N=number of samples; F=PLS factor number; R^2 coefficient of determination; RMSEC=root mean square error of calibration; Cal = calibration; CV = cross-validation; SECV Standard error cross-validation; SEP=standard error of prediction.

Table 7. Discriminant model prediction statistics for determination of chlorpyrifos-methyl residues based on the DA7200 analogue for selected wavelengths[a] of the NIR LED-based Prototype 2 instrument.

Model Data	Calibration			Independent Validation		
	False Positive	False Negative	Overall % CC	False Positive	False Negative	Overall % CC
Rough Rice (Low: ≤ 3.0 ppm, High: >3.0 ppm)						

ALLQualRR	5/135	8/135	90.4	-	-	-
CL151	3/108	6/108	91.7	2/27	3/27	90.7
Diamond	0/108	5/108	95.4	4/27	5/27	83.3
Hybrid1	1/108	4/108	95.4	0/27	4/27	92.6
Gemini	2/108	7/108	91.7	5/27	2/27	87.0
Hybrid2	6/108	4/108	90.7	1/27	1/27	96.3
Brown Rice (Low: ≤ 1.5 ppm, High: >1.5 ppm)						
ALLQualBR	27/134	4/135	77.0	-	-	-
CL151	13/108	5/108	83.3	13/26	5/27	71.3
Diamond	19/107	1/108	81.5	6/27	6/27	77.8
Hybrid1	19/107	4/108	78.7	9/27	3/27	77.8
Gemini	17/107	2/108	82.4	10/27	2/27	77.8
Hybrid2	17/107	4/108	80.6	8/27	4/27	77.8
Milled Rice (Low: ≤ 0.2 ppm, High: >0.2 ppm)						
ALLQualMR	0/135	0/135	100.0	-	-	-
CL151	0/108	0/108	100.0	0/27	0/27	100.0
Diamond	0/108	0/108	100.0	1/27	1/27	96.3
Hybrid1	0/108	0/108	100.0	0/27	1/27	98.2
Gemini	0/108	0/108	100.0	0/27	0/27	100.0
Hybrid2	0/108	0/108	100.0	0/27	0/27	100.0

[a] Wavelengths at 80% relative radiant intensity for Prototype 2 includes 970-985 nm, 1035-1060 nm, 1185-1215 nm, 1275-1320 nm, 1420-1470 nm, 1515-1580 nm, 1555-1630 nm, and 1620-1680 nm; % CC=percent correct classification.

For the PLS analysis, across the five one-variety out models for rough rice, the R^2 for validation sets ranged from 0.59 to 0.74 with SEP ranges of 2.24 to 2.85, brown rice had R^2 of 0.74 to 0.78 and SEP of 1.02 to 1.12 and milled rice had R^2 of 0.67 to 0.82 and SEP of 0.14 to 0.17, Table 6. Discriminant analysis showed that the overall %CC for validation sets predicted from one-variety-out calibration models had good potential for applications for rough rice at %CC of 83.3 to 96.3 and milled rice at 96.3 to 100 %CC while brown rice was lowest at 71.3 to 77.8 %CC. The lower performance for brown rice may be attributed to the higher complexity of the constituents in this rice form especially when compared to milled rice where bran has already been removed. Considering that Dors et al. [11] showed that the highest concentrations of pesticide residues were found in the bran fraction, it becomes even more critical that better prediction capabilities are targeted for brown rice. However, the larger market and supply of rice for consumption in the Philippines and many other countries remain to be milled rice. With the potential 96% to 100% correct classification for detection of milled rice containing low versus high levels of CMPR, the use of NIR multi-spectra LED instrument presents an additional viable technique that will readily assist in ensuring food safety for the rice industry.

5. Conclusions

The potential for detecting the level or concentration of chlorpyrifos-methyl pesticide residues in rough, brown, and milled rice using a NIR LED-based instrument that uses selected wavelengths (red for ~700 nm as a power indicator and NIR wavelengths of 980, 1050, 1200, 1300, 1450, 1550, 1600, and 1650 nm) was shown. Based on DA7200 analogue, the averaged quantitative prediction statistics for the five one-variety out models for the proposed LEDPrototype2 will be able to predict rough rice at an R^2 of 0.64 and SEP of 2.62, brown rice at an R^2 of 0.76 and SEP of 1.05, and milled rice at R^2 of

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0.74 and SEP of 0.15. Simulations that are likewise DA7200 analogue for qualitative predictions using five one-variety out models showed an average percent correct classification of 90.0 for rough rice, 76.5 for brown rice, and 98.9 for milled rice. These findings provided concrete basis to pursue the design and development of a small tabletop or handheld device (LEDPrototype2) that incorporates the use of the selected important wavelengths into the basic design concept of LEDPrototype1 while improving the signal and noise considerations and working within constraints such as LED availability, cost, simplicity in operation, and availability of components so that it can be fabricated in specific target locations, such as the Philippines. LEDs are low-cost options for rapid multispectral measurements and instrumentation that measure or differentiate low and high concentrations of pesticide residues in rice will be highly beneficial to the rice industry, safety regulators, and consumers.

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References

1. Codex Alimentarius Commission. Report of the 49th Session of the Codex Committee on Pesticide Residues. Joint FAO/WHO Food Standard Programme Codex Alimentarius Commission. 2017. 40th Session. REP17/PR. Viale delle Terne di Caracalla, 00153 Rome Italy. Retrieved from www.codexalimentarius.org.
2. European Food Safety Authority. Statement on the available outcomes of the human health assessment in the context of the pesticides peer review of the active substance chlorpyrifos-methyl.ej EFSA Journal. Retrieved from www.efsa.europa.eu/efsajournal. 2019. 23pp.
3. Mie, A., Ruden, C., & Grandjean, P. Safety evaluation of pesticides: Developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl. Environ. Health, 2018. 17(1), 77. doi: 10.1186/s12940-018-0421-y.
4. Philippine Daily Inquirer. NFA: 330,000 bags of imported rice infested with weevils. 2018. Retrieved from https://newsinfo.inquirer.net/1024529/nfa-330000-bags-of-imported-rice-infested-with-weevils.
5. Rodriguez, F.S., Armstrong, P.R., Maghirang, E.B., Yaptenco, K.F., Scully, E.D., Arthur, F.H., Brabec, D.L., Adviento-Borbe, A.D., & Suministrado, D.C. (2020). NIR spectroscopy detects pesticide residues containing chlorpyrifos-methyl in rough, brown, and milled rice. Trans. ASABE, accepted May 2020.
6. Pearson, T., Maghirang, E., & Dowell, F. A multispectral sorting device for wheat kernels. 2013. Amer. J. Agric. Sci. Tech., 2, 45-60.
7. Shenk, J.S., Workman, Jr., J.J., & Westerhaus, M.O. Application of NIR spectroscopy to agricultural products. In Handbook of Near-Infrared Analysis, Burns, D.A. & Ciurczak, E.W. (eds), 1992 (pp. 383-431). New York, USA: Marcel Dekker, Inc.
8. Workman, Jr., J., & Weyer, L. Practical Guide to Interpretive Near-Infrared Spectroscopy. 2008. Boca Raton, Florida, USA: CRC Press Taylor & Francis Group.
9. Marubeni America Corporation, Silicon Valley Branch. Datasheet. 3945 Freedom Circle, Suite 1000 Santa Clara, CA 95054 USA. Retrieved from https://tech-led.com/en/product/ir-nir-leds/ on 2019
10. Williams, P.C. & Norris, K. Near-Infrared Technology in the Agricultural and Food Industries, 2nd ed. 2001. St Paul, MN: American Association of Cereal Chemists, Inc.

 doi:10.20944/preprints202405.0188.v1

11. Dors, G.C., Primel, E.G., Fagundes, C.A.A., Mariot, C.H.P., & Badiale-Furlong, E. Distribution of pesticide residues in rice grain and in its coproducts. J. Brazil. Chem. Soc., 2011. 22(10), 1921-1930.
12. Delwiche, S.R. High-speed bichromatic inspection of wheat kernels for mold and color class using high-brightness pulsed LEDs. Sens. Instrum. Food Qual. Saf.,2008. 2,103-110.
13. Ding, J., Sun, X., Guo, Y., Jia, H., Qiao, L., & Wang, X. A portable pesticide residues detection instrument based on impedance immunosensor. Sensors & Transducers,2014. 172(6), 27-33.
14. Lin, L., He, Y., Xiao, Z., Zhao, K., Dong, T., &Nie, P. Rapid-detection sensor for rice grain moisture based on NIR spectroscopy. Appl. Sci.,2019. 9(8), 1654, 13pp.
15. Perten Industries. Diode Array 7250: At-line and Lab NIR analysis system. www.perten.com, DA7250 Brochure, 6 pp.
16. Stasiewicz, M.J., Falade, T.D.O., Mutuma, M., Mutiga, S.K., Harvey, J.J.W., Fox, G., Pearson, T.C., Muthomi, J.W., & Nelson. R.J. Multi-spectral kernel sorting to reduce aflatoxins and fumonisins in Kenyan maize. Food Cont.,2017. 78, 203-214.
17. Yeh, T.S., & Tseng, S.S. A low cost LED based spectrometer. J. Chinese Chem. Soc., 2006. 53(5), 1067-1072.