2020

PhilRice R&D Highlights

Rice Chemistry and Food Science Division

Contents

Rice Chemistry and Food Science Division

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EXECUTIVE SUMMARY

The Rice Chemistry and Food Science Division (RCFSD) supports the goal of increasing the productivity and profitability of rice farming systems by determining grain quality (GQ) characteristics of rice, developing technologies on other uses of rice and its by-products, and promoting these high-quality and value-added products to benefit consumers/farmers and food manufacturers. To accomplish this, four core and two extra-core funded projects were implemented in 2020. The core-funded projects were: (1) Centralized screening for grain quality; (2) Nutrition, health, and wellness potential of Philippine rice and rice-based crops; (3) Quality improvement on the laboratory management of the RCFSD; and (4) Rice quality and safety: Method development and detection. The extra-core projects were: (1) Red mold rice as a natural dietary supplement: Market studies funded by the DA-BIOTECH and (2) Improving brown rice quality, shelf life, and engineering technologies funded by the DA-Bureau of Agricultural Research. The core-funded Projects 1 and 4 aimed to provide reliable data on rice grain quality characteristics to ensure that rice available to the different stakeholders has good grain quality and is safe for consumption. The core-funded Project 2 and the two extra-core projects focused on developing technologies on other uses of rice and its by-products with enhanced value for better quality, health, nutrition, and income, backed by the information on GQ and other quality characteristics of these materials. Lastly, the core-funded Project 3 ensured that all these data generated in RCFSD laboratories are accurate through quality management compliant with national and international standards.

The Division received 1,830 rice samples for GQ analysis under its Centralized GQ screening project. These samples comprised pre-National Cooperative Testing lines and other rice samples, rice germplasm collections/accessions, and cytoplasmic male sterility-based parental lines and F1 hybrids. Of this number, 1,082 samples were evaluated for GQ, and 479 rice germplasm data were submitted for inclusion in the Germplasm Management System. These results contributed to enhancing the breeding of rice with excellent GQ and delivering grain quality data responsive to the needs of its target users.

With the huge number of samples evaluated yearly for GQ parameters, there is a need for rapid and reliable methods of GQ evaluation. Thus, Project 4 was implemented. In this project, the use of the microplate reader and Near-infrared

EXECUTIVE SUMMARY

Reflectance Spectrophotometer (NIRS) were explored for physicochemical properties determination for subsequent routine use. The use of a microplate reader for amylose content (AC) estimation was optimized based on the conventional iodine-binding colorimetric method. It had higher than 92% accuracy for qualitative measurements of AC, was from 13 to 16 times faster than the conventional method, and generated 20 times fewer wastes. NIRS calibration models built with flour samples were better than those for milled rice. The models could simultaneously and non-destructively predict the moisture content (MC) and protein content values of unknown samples with 97% and 94% accuracy, respectively, at 80-100 samples per day. Further enhancements are being conducted to ensure the accuracy and reliability of the methods for routine testing of numerous samples. In addition to enhancing the overall efficiency of the GQ evaluation process, this project contributed to advancing rice science by improving or updating methods for routine grain quality testing.

To accomplish the objective of Project 4 on ensuring that the rice available for consumers are of good GQ and are safe, six rice samples collected from different National Food Authority warehouses in Albay, Negros Occidental, Isabela, Cagayan de Oro, and Tarlac were evaluated for their GQ and pesticide and heavy metal contamination. Generally, the GQ properties were comparable, and their pesticide residues and heavy metal content were all below the maximum residue limit.

Under Project 2, local rice and rice-based crops were evaluated as potential sources of nutrition, health, and wellness for consumers. Peptides from rice wine lees, or the residue of rice wine fermentation, were derived and evaluated for their antioxidant activities. The 5-10kDa peptides displayed the highest ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, followed by >10kDa and <5kDa fractions. For 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity, the antioxidant power of 5-10 kDa and <5kDa peptides were significantly higher than that of the >10kDa fractions. Flour forms of raw polished rice (4 modern non-pigmented and two traditional pigmented) varieties were also evaluated for *in vitro* starch digestibility and *in vitro* glycemic index (GI). Resistant starch (RS) values of polished rice were low (0.1% to 0.7%), but Ominio and Dinorado had increases in RS content from 0.1% (polished) to 2.68% and 2.29%, respectively, when unpolished. The high AC-intermediate gelatinization temperature (GT) variety NSIC Rc 282 had moderate estimated GI, while the waxy variety Ominio was classified as high-GI. Lastly, the bran of NSIC Rc 160, Dinorado (red rice), and Ominio (purple rice) varieties were fermented by a combination of solid-state and submerged liquid fermentation systems using fungi and lactic acid bacteria, and the resulting products had generally higher total phenolic, flavonoid, and anthocyanin content than their nonfermented counterparts. Despite these increases in phytochemical content, no significant effect on the samples' chemical compositions was generally obtained.

In addition to the evaluation of health-promoting properties of rice and their by-products, Project 2 also looked into consumer awareness on and demand for low-GI foods to provide insights on developing interventions, such as rice and rice-

based products with low GI and dietary/health supplements, for the management of diabetes among target populations. Diabetes is a non-communicable disease that has afflicted Filipinos over the years. The online survey conducted among 328 respondents (≥18 years old, 123 males, 205 females) from all 16 regions in the country showed that both male and female respondents indicated that they manage their diabetes primarily by eating healthy foods; some take dietary/health supplements. The majority (60%) were aware of GI, and many were willing to buy rice with naturally low GI, brown rice, and cereals. More than half of both sexes were also interested in low-GI rice-based products, notably bakery foods, *kakanin*/native rice cakes, and ready-to-drink beverages, if these were available in the market.

For the two extra-core funded projects, the marketability of red mold rice (RMR) as a functional ingredient or dietary supplement and the improvement of the quality and shelf life of brown rice was determined. In the first project, a survey of 30 local pharmaceutical companies revealed no existing RMR product in the country. Thus, an RMR dietary supplement was developed. It was either superior or comparable with other RMR-based supplements in terms of total phenolic content and antioxidant activity (DPPH, ABTS, and FRAP). It also contained higher cholesterol-lowering compound mevinolin than other commercially available products and was safe for intake as it had a low citrinin level, which meets international standards. In the second project, the quality of brown rice stabilized through the optimized steam heating process and stored in different packaging materials at a cooler temperature (~4-10°C) was similar to that of the unstabilized samples in terms of microbial content and physicochemical and sensory properties. Still, the stabilized rice bran developed lower free fatty acids and lipase activity after four months of storage. FFA and lipase activity are indicators of hydrolytic rancidity in foods, and should these results endure as the storage time progresses, the optimized stabilization process could be a promising technique for lengthening the shelf life of brown rice.

Finally, to support all these research projects and comply with the Integrated Management System of the Institute, the core-funded Project 3 was implemented. In this project, calibration, validation, and preventive maintenance of critical laboratory equipment and devices were performed. An automated chemical inventory system was developed for safer and more efficient chemical supplies monitoring. Moreover, chemical wastes were treated based on the established waste treatment protocol, and all regulatory requirements for controlled chemicals were submitted. Lastly, laboratory personnel were capacitated through five in-house training activities on calibration and use of new sophisticated laboratory equipment.

All these accomplishments were done at the height of the coronavirus pandemic. Achievement of some project targets was hampered or delayed due to the lockdowns. With this challenge, the following are underscored: development and adoption of rapid and reliable methods for routine GQ testing need to be fast-tracked, and research on the health-promoting and immunity-boosting properties of rice and by-products must be intensified.

Centralized Screening for Grain Quality

Evelyn H. Bandonill

Through time, breeding activities have heightened, and demand for fast and reliable information on rice grain quality has increased significantly. Available information in the PhilRice Genebank on the morphological and agronomic traits, reaction to biotic and abiotic stresses, and grain quality characteristics are lacking. Moreover, a simple yet efficient screening for grain quality is an integral component of an effective breeding program for hybrids. Centralization of grain quality (GQ) evaluation of rice samples from various sources/ breeding groups will therefore ensure accuracy and repeatability of results such that data are ready for the breeders before the next planting season. To continue supporting the development of high-yielding rice varieties for both inbred and hybrid breeding and the generation of GQ descriptors of the PhilRice germplasm collections/ accessions through grain quality screening, this project was implemented. In 2020, a total of 674 pre-National Cooperative Testing lines and other rice samples in 2019 Dry Season and Wet Seasons and 2020DS were evaluated for grain quality. A total of 34 entries exhibiting good grain quality were identified and recommended to the breeders, which will help in their selection process.

Moreover, from the 504 rice germplasm collections/accessions received for GQ evaluation, 214 samples were subjected to complete GQ analysis, which identified eight samples that passed the GQ standards and predicted 237 entries to have tender cooked rice texture based on amylose content-gelatinization temperature combination. This also led to the submission of available GQ data of 479 samples in the Germplasm Management System.

Lastly, out of the 652 cytoplasmic male sterility-based parental lines and F1 hybrids with complete GQ data, 27 hybrid lines were recommended to the breeders for their hybrid rice selection. Moreover, a list of 212 entries was made, which predicted the hybrid entries to have a tender cooked rice texture. These study results contributed to the desired outcome of the project that is enhanced breeding with excellent grain quality and enhanced delivery of grain quality data to its target users.

Grain Quality Screening of Pre-NCT Rice Lines

Amelia V. Morales, Evelyn H. Bandonill, and Raffy B. Rodriguez

Grain quality evaluation plays an important role in the rice breeding program. Preference for grain length and shape, similar to low to intermediate amylose content (AC), may vary from one group to another, but a higher value is assigned for long to extra-long grain length and slender shapes. In contrast, the presence of chalk in the rice grain has been described as a defect that affects milling, marketing, and storage properties. The pre-National Cooperative Testing lines are evaluated and screened for grain quality (GQ) to trim down the number of lines advancing for further trials. This year, 756 rice samples were received for GQ analysis from the Plant Breeding and Biotechnology Division and Genetic Resources Division. These came from different groups, namely: Rainfed lowland/adverse environment (Rainfed), hybrid rice, irrigated lowland-direct seeded rice, preliminary yield trial-special purpose, Preliminary Yield Trial-Transplanted (PYT-TPR), and PYT-TPR Flooded-Drought. It likewise included Ifugao, Lake Sebu, NSIC Rc 60, and NSIC Rc 300 rice varieties. The 307 received samples were completely analyzed based on the breeders' requested parameters. Among the rainfed and hybrid rice lines, the majority met the GQ standards for milled and head rice recovery, number of immature grains, grain length, and shape. However, few entries passed the standards for chalky grains, AC, and gelatinization temperature. Eight hybrid rice lines were subjected to sensory evaluation wherein all raw samples had no aroma and were generally slightly white, dull to slightly glossy, with 41% to 60% and 61% to 80% chalky grains and hard to slightly hard grains. In the cooked form, all entries had no aroma and bland and were generally white, glossy, slightly cohesive, slightly tender to tender, and slightly smooth to smooth. The grain quality evaluation allows the identification of lines or other rice samples with properties suited for special purposes, thus reducing the cost of analyses and maximizing the resources in the rice breeding program.

Evaluation of PhilRice Germplasm Collection for Grain Quality

Amelia V. Morales, Xavier Greg I. Caguiat, Marilyn C. Ferrer, Evelyn H. Bandonill, and Raffy B. Rodriguez

Aside from yield, resistance to pests and diseases, and agro-morphological characteristics, grain quality, which are also influenced by genotype, an important role in the rice breeding program. Long to extra-long grain length and slender shapes are highly preferred, similar to those having low to intermediate amylose content (AC) that is expected to result in tender cooked rice with good eating quality. This year, 504 rice germplasm were received, and 498 were analyzed for GQ. The majority of the 214 rice germplasm evaluated for milling recovery and physical properties met the standards for brown rice, milled and head rice recovery, and rate of immature grains. However, only a few entries passed the standards for chalky grains, grain length, grain shape, and gelatinization temperature. Among the entries, two samples with collection numbers 1703 and 7394 passed all the standards for GQ except for AC, which turned out to be high. Among the entries with waxy to very low AC (glutinous-type), six samples with collection no. 2148, 10882, 16248, 16263, 16742, and 16473 passed all the standards for milling recovery and physical attributes. Of the 479 samples completely evaluated for AC and gelatinization temperature (GT), 84 rice germplasm passed both the preferred intermediate classification for AC and GT. Meanwhile, 237 were predicted to have a tender texture when freshly cooked based on their AC and GT combination. The data generated provided important information through a computerized database system in the rice breeding program maximizing the use of rice germplasm as well as utilization for other purposes.

Screening of CMS Parentals, Breeding Lines, and Promising Hybrids for Grain Quality

Evelyn H. Bandonill, Jay Carl A. Cacerez, Lynnden C. Castillo, Frodie P. Waing, and Joanne D. Caguiat

Increased availability of cytoplasmic male sterile (CMS) parentals, breeding lines, and hybrids can be achieved through early grain quality screening aside from pest and disease resistance screening. This can significantly contribute to the fast turnaround of results by providing the data to the breeders earlier for their use before the next planting season. To identify CMS parentals, breeding lines, and promising hybrids with acceptable grain quality, 166 usable, advance CMS-based parental lines, and F1 hybrids; 53 usable and advanced CMS-based parentals; and 433 advanced CMS-based parental lines from the 2019 WS were evaluated. Out of the 166 usable advanced CMS-based parental lines and F1 hybrids, 37%, 27%, and 57% passed the standards for milling, physical, and physicochemical properties, respectively. Four maintainers, six restorers, and one elite line were identified for use in the breeding program when their yield performance and pest and disease resistance are also considered. From the 53 usable and advanced CMS-based parentals, 53% had good milling potentials while 72% had acceptable amylose content-gelatinization temperature (AC-GT) combination (L-L, L-I, I-I/HI, I-L). Among the 433 advanced CMS-based parental lines, 212 entries were identified promising based on AC-GT combination and were clustered as follows: low AC - low GT (126 entries), low AC - intermediate/high intermediate (HI) GT (9 entries), intermediate AC - intermediate/HI GT (77 entries), indicating tender cooked rice.

PROJECT 2

Nutrition, Health, and Wellness Potential of Philippine Rice and Rice-based Crops

Riza G. Abilgos-Ramos

Non-communicable illnesses (cancers, diabetes, and cardiovascular diseases) are still the top causes of death in the country. As a result, the demand for wellness products and functional food is increasing. More consumers are nutrition-insecure as wasting and stunting among children remain a public health concern. This project assessed the potential of local rice and rice-based crops as sources of nutrition, health, and wellness for consumers. First, rice wine lees' health-promoting properties were evaluated. Second, consumer awareness on and demand for low-glycemic index (GI) foods were determined, and then *in vitro* GI of modern and traditional rice varieties were measured before the potential health benefits of fermented rice bran were assessed. Results showed that 5-10kDa peptides extracted from rice wine lees exhibited the highest ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, followed by >10kDa and <5kDa fractions. For 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging activity, the antioxidant power of 5kDa to 10kDa and <5kDa peptides were significantly higher than that of the >10kDa fractions. Moreover, the pH condition during enzymatic hydrolysis significantly affected the antioxidant activity of peptide fractions. Meanwhile, 60% of the online survey respondents (≥18 years old, 123 males, 205 females) were aware of low-GI foods and were interested or willing to try low-GI rice and rice-based products. Unpolished Dinorado and Ominio were found to have a 20 times higher amount of resistant starch than the polished samples. The estimated GI of NSIC Rc 282 and Ominio were medium and high, respectively. Rice bran fermentation of NSIC Rc 160, Dinorado (red rice), and Ominio (purple rice) resulted in a higher total phenolic and flavonoid content than the control (except for the anthocyanin content of Ominio). Fermented rice bran samples also showed higher crude fiber, moisture, and ash content, and lowered crude fat content. Thus, fermentation can enhance the extractability of bioactive compounds and increase the phytochemicals and antioxidants activity of rice bran. Findings from these studies show the potential of rice as a source of health-promoting and disease-preventing compounds, while the information on consumer knowledge and demand for low-GI foods provide relevant market information for product developers and the food industry.

Utilization of Rice Wine Lees for Food and Feed

Henry M. Corpuz, Rosaly V. Manaois, Amelia V. Morales, and Lynnden C. Castillo

Cognitive impairment and dementia are recognized as one of the health problems worldwide because of their contributions as risk factors for illness and mortality among the elderly. These neurodegenerative diseases are associated with amyloid plaque accumulation, tau protein aggregation, neuroinflammation, cholinergic dysfunction, and cerebral oxidative stress. The management of these mental conditions entails a serious financial, personal, and societal burden. Therefore, there is a need to search for food components with biologically active molecules to prevent age-related cognitive impairment progression. Rice wine lees (RWL) is a by-product of rice wine production, and large quantities of this residue are discarded as industrial waste. It has been reported that RWL contains high levels of fiber and protein, making it a viable source of bioactive peptides with health-promoting properties. However, the potential health benefits of RWL bioactive peptides have not yet been investigated. This study aimed to evaluate the antioxidant activity and neuroprotective property of bioactive peptides derived from RWL against oxidative stress-induced cytotoxicity in human neuronal cells. RWL crude protein extract was hydrolyzed by enzymatic digestion using 0.05% (v/v) of protease from *Bacillus licheniformis*. RWL protein hydrolysates were dialyzed with 500Da to 100Da membrane to remove soluble sugars and other impurities and separated by ultrafiltration using 10kDa and 5kDa membranes. The antioxidant activities of three peptide fractions (>10, 5-10, <5kDa) were also determined. Results showed that 5-10kDa peptides exhibited the highest ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, followed by 10kDa and <5kDa fractions. For 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity, the antioxidant power of 5kDa to 10kDa and <5kDa peptides were significantly higher than that of the >10kDa fractions. Moreover, the pH condition during enzymatic hydrolysis significantly affected the antioxidant activity of peptide fractions. The evaluation of bioactive peptide fractions' neuroprotective properties is ongoing to assess their ability to inhibit the oxidative stress-induced toxicity in SH-SY5Y neuronal cells.

Glycemic Index of Commonly-Grown Rice in The Philippines

Rosaly V. Manaois, Riza G. Abilgos-Ramos, Raffy B. Rodriguez, Xenia Portia S. Fuentes, and Alcel B. Atanacio

Diabetes is a leading cause of mortality among Filipinos, and its prevalence has been increasing. Dietary interventions, particularly the consumption of foods with a low glycemic index (GI), could help curb this health problem. GI is a measure of the blood glucose-raising potential of food compared to a reference food (generally pure glucose). This study was conducted to establish consumer knowledge and demand for low-GI food and provide data on the GI of commonly grown local rice. An online survey was conducted to determine consumer knowledge of GI. The majority (>60%) of the 328 respondents (≥18 years old, 123 males, 205 females) from all regions in the country had a high level of GI awareness. Although only 4% of them had pre-diabetes/diabetes and 13% have a family history of the disease, all respondents signified interest in rice with naturally low GI. The majority were willing to buy low-GI rice, brown rice, and cereals. More than half were also interested in low-GI rice-based products, notably bakery foods, *kakanin*/native rice cakes, and ready-to-drink beverages. This initial study is relevant for the food industry as it provides market demand information for the targeted consumers. For the evaluation of an *in vitro* method of GI analysis, selected varieties were tested. The resistant starch (RS) fractions of the polished samples were low (0.1% to 0.7%), but the RS of unpolished Dinorado and Ominio were higher by 20 times. The estimated GI (eGI) of NSIC Rc282 and Ominio were moderate and high, respectively. Other samples recorded high eGI, regardless of AC and GT. Further evaluation of the method is being carried out to improve accuracy and precision and make it suitable for a higher number of samples. Partnership with a capable clinical research institution is also being sought for the *in vivo* GI determination.

Fermented Rice Bran for Functional Product Development and Health Improvement

Henry M. Corpuz, Rodel M. Bulatao, Amelia V. Morales, Rochelle C. Huliganga, and Maricar B. Castillo

Rice bran, an abundant and underutilized by-product of the rice milling process, is a rich source of bioactive compounds. Recent studies have shown that fermentation can improve their biological activities. This study aimed to evaluate the potential health benefits of fermented rice bran for functional food product development to improve the health status of adult populations.

Fermented rice bran (FRB) of NSIC Rc 160, Dinorado (red rice), and Ominio (black rice) were prepared using the optimized protocol. Rice bran samples were fermented by a combination of solid-state and submerged liquid fermentation systems using fungi and lactic acid bacteria. The control or non-FRB was prepared following the same procedure except for the addition of fermenting microorganisms. FRB samples and their unfermented counterparts were characterized for their phytochemicals, antioxidant activities, microbial load, and nutritional quality. All FRB samples showed higher total phenolic and flavonoid content than the control, except for the anthocyanin content of the Ominio variety. The increase in phytochemical levels of FRB ranged from 27% to 57%.

Moreover, the ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) activities of all FRB samples were higher than that of the control by 22% to 33%. Higher total aerobic plate count was detected in FRB samples than control, except for Ominio variety. Although FRB samples showed higher crude fiber, moisture, and ash content and lower crude fat content than the control group, fermentation did not significantly change the samples' proximate compositions. Results suggest that the fermentation process could enhance the extractability of bioactive compounds and increase the phytochemicals and antioxidants activity of rice bran. FRB could be a potential source of bioactive compounds with disease-preventing and health-promoting benefits.

PROJECT 3

Quality Improvement on the Laboratory Management of the Rice Chemistry and Food Science Division

Rodel M. Bulatao

For more than 25 years, the Rice Chemistry and Food Science Division (RCFSD) continues to deliver quality outputs through intensive research and strong linkages with the private sector and other research agencies. Good laboratory management has been one of the key factors in the delivery of high-quality output and services. This year, the existing RCFSD Laboratory Manual was updated through consolidation and harmonization of all test methods and protocols to generate consistent, accurate, and reproducible data among the analysts. As one of the research divisions, RCFSD houses around 40 state-of-the-art laboratory equipment and analytical instruments, which require periodic preventive maintenance, calibration, and validation for optimum performance, longer service, and prevention from further damage and deterioration. In 2020, 18 laboratory equipment and devices (e.g., orbital shaker, UV-Vis spectrophotometer, and centrifuge) were calibrated by an external service provider, while 19 equipment (e.g., analytical balances, ovens, and refrigerators) were attuned by in-house trained calibrators, as indicated in the RCFSD calibration plan. The validation (e.g., pipettors and balances) and preventive maintenance of some analytical equipment (e.g., Ultra-performance liquid chromatography, high-performance liquid chromatography, water purification system, and protein analyzer) were also performed. Meanwhile, about 1,650L of chemical wastes generated from amylose (1,260L) and protein (390L) analyses were treated and disposed of following the established protocols for chemical waste treatment developed in 2019. Chemical management was also made easy by developing a fast and cost-effective automated chemical inventory system (ACIS). ACIS was designed to monitor the real-time addition (entry) and deduction (withdrawal) of chemicals in and out of the stockroom. Furthermore, in compliance with the Philippine Drug Enforcement Agency (PDEA), a list of PDEA controlled chemicals (e.g., acetone, hydrochloric acid, and sulfuric acid) consumed and stored by RCFSD were consolidated and submitted to the Procurement Management Division. Lastly, to continuously improve the skills and competitiveness of laboratory personnel, five in-house training activities on basic operation and troubleshooting of newly acquired equipment and one calibration training on balances and enclosures were conducted.

PROJECT 4

Rice Quality and Safety: Method Development and Detection

Henry M. Corpuz

Grain quality (GQ) is one of the important considerations in the rice breeding program. GQ consists of the milling potentials, physical attributes, physicochemical properties, cooking parameters, and sensory qualities of rice and is routinely analyzed to assess the marketability, consumer acceptability, and palatability of promising germplasms. From consumers' perspective, starch physicochemical properties matter as they are key indicators of rice cooking and eating quality. Some of the most important rice physicochemical properties include amylose content (AC), gelatinization temperature (GT), and protein content (PC). Amylose is the primary determinant of cooked rice texture, while GT estimates the time needed to cook the grains. These parameters are measured in the laboratory by conventional chemical methods that are laborious and time-consuming, require enormous resources (e.g., effort, time, supplies), and generate a large volume of toxic chemical wastes. The RCFSD is tasked to evaluate the GQ of rice breeding materials/lines developed by PhilRice breeding groups and rice entries submitted by various breeding institutions through the National Cooperative Testing Program. Our team receives more than 1,000 samples annually for GQ analysis. Therefore, rapid, cost-effective, safe, and accurate alternative methods for measuring rice physicochemical properties are necessary to fast-track the evaluation process. Moreover, the need for a new method for GQ evaluation is recognized under "Advanced rice science and technology as continuing sources of growth."

In this project, robust microplate reader and near infrared reflectance spectroscopy (NIRS)-based assays were adopted to provide fast and reliable methods for routine analysis of rice physicochemical properties. The use of a microplate reader for AC estimation was optimized based on the conventional iodine-binding colorimetric method. The efficiency was improved by lowering the amounts of reagents and reducing the times in certain steps. The optimized microplate-based AC assay involved the addition of 100µL of 95% ethanol and 900µL of 1N NaOH into 10mg rice flour, heating the mixture at 99°C to 100°C for 5min and mixing with 9mL of distilled water, dilution of 15µL aliquot with 13x water in a microtiter plate, addition of 30µL of 0.09N NH_4Cl and 60µL of 0.015% I_2/KI, and reading at 620nm in a microplate reader. The optimized protocol had higher than 92% accuracy for qualitative measurements of AC using samples with known and unknown AC values. Analysis was 13 to 16 times faster than the conventional method, and the number of wastes generated was reduced twentyfold.

NIRS offers a fast, easy-to-use, and nondestructive analytical technique. Hence, we calibrated the NIRS machine acquired in 2018 to predict the AC, MC, and PC

of rice samples. Using modified partial least-squares (PLS) regression, calibration models were developed from 580 milled rice and 470 flour samples representing a wide range of reference values. Results showed that calibration models built with flour samples are better than milled rice, as indicated by the higher coefficient of determination (RSQ) and lower standard error of calibration (SEC) and prediction (SEP). Moreover, PC and MC calibration models for flour sets were superior to the AC calibration model. The validation test showed that the developed models could predict the MC and PC values of unknown samples with 97% and 94% accuracy, respectively. The NIRS-based assay could expedite the evaluation of rice's physicochemical properties because the machine can simultaneously analyze the PC and MC of around 80 to 100 samples per day. Also, the generation of toxic chemical waste from conventional protein analysis was completely eliminated.

Food safety is another element of quality. Food is safe if it does not contain any contaminants that might compromise consumers' health. With the anticipated growth in international rice trade, consumers in many countries are exposed to various food-borne illnesses caused by toxic compounds present in the grains. Hence, food safety measures are needed to ensure that commercial all rice, whether locally produced or imported, are free from heavy metals, high levels of pesticides, pests, and food-borne microorganisms. This year, six rice samples were collected from different National Food Authority (NFA) warehouses in Albay, Negros Occidental, Isabela, Cagayan de Oro, and Tarlac and evaluated for their GQ, heavy metal content, and pesticide residue level. Results of the GQ evaluation showed that the samples had 32.1% to 52.6% broken and 12.7% to 25.6% chalky grains. Local rice samples were long and intermediate, while the imported rice had long and slender grains. Moisture content was generally lower than the declared values (12% to 14%). Local rice samples had high AC with intermediate GT, while a sample imported from Pakistan had intermediate AC and low GT. Protein content ranged from 6.4% to 7.2%. Sensory analysis showed that all raw samples had neither pleasant aroma nor off-odor with different whiteness, gloss, translucency, and hardness. In contrast, cooked samples were bland, slightly glossy, slightly tender, slightly smooth, and had no aroma, off-odor, or off-taste.

Chemical contaminant analyses showed that the organochlorine and organophosphorus pesticide levels detected from all samples were below the maximum residue limit (MRL) for rice. The samples also passed the MRL for arsenic, cadmium, lead, and mercury. A preliminary study was also conducted to assess NFA rice samples' spoilage rate. Cooked rice samples stored at room temperature for 24h had a higher microbial load than those stored at cold temperatures. Six out of ten samples had a total plate count of 1.15 to 27.70 $x10^7$ CFU/g, resulting in lower sensory index/acceptability due to the perceived off-odor of spoiled leftover rice. Refrigerated samples remained acceptable even after 24h of storage.

In conclusion, the microplate-based assay can be used for AC determination of early generation rice lines. Further enhancements are being conducted to ensure the accuracy and reliability of the procedure, with a faster turnaround of results suitable for the routine testing of numerous samples. NIRS machine can be utilized for rapid and nondestructive screening of rice PC and MC. However, refinement or

updating of the calibration models is needed to further improve the prediction accuracy and reliability of the machine for routine screening of large rice samples. Lastly, the NFA rice samples generally had comparable GQ characteristics, whether locally grown or imported. All six rice samples collected from NFA warehouses were relatively safe as their pesticide residues and heavy metal contents were below the MRL. However, the result of pesticide residue analysis must be validated using a more sensitive instrument.

Development of Rapid Methods of Amylose Content Determination

Rosaly V. Manaois and Jay Carl A. Cacerez

Amylose content (AC) is an important property in developing new rice varieties and selecting appropriate varieties for various processing applications. The conventional iodine-binding colorimetric assay of Juliano et al. (2012) is employed to evaluate these rice lines. However, to meet the increasing demands of researchers for AC analysis, a faster and higher throughput method is needed, at least for the early generation samples. This study was therefore conducted to develop fast and reliable methods for routine AC analysis. This year, a microplate reader for AC estimation was optimized based on the conventional AC method. The efficiency was improved by lowering the amounts of reagents and reducing the times in certain steps. The optimized microplate-based AC assay involved the addition of 100µL of 95% ethanol and 900µL of 1N NaOH into 10mg rice flour, heating the mixture at 99°C to 100°C for 5min and mixing it with 9mL of distilled water, dilution of 15µL aliquot with 13x water in a microtiter plate, addition of 30µL of 0.09N NH_4Cl and 60µL of 0.015% I_2/KI, and reading at 620nm in a microplate reader. The procedure yielded higher than 92% accuracy for qualitative measurements when tested using samples with known and unknown AC values. Analysis was 13 to 16 times faster than the conventional method, and the number of wastes generated was 20 times lower. In conclusion, this method is an environment-friendly and more economical means to provide useful and timely AC information for early generation rice lines. Further enhancements are being conducted to ensure the accuracy and reliability of the procedure, with a faster turnaround of results suitable for routine testing of numerous samples.

PROJECT 4

Grain Quality and Safety Assessment of Local and Imported Milled Rice in the Philippines

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As the government necessitates importing rice from neighboring countries to meet the country's declining buffer stock, every Filipino's right to access safe and high-quality rice remains a top priority. This study aimed to generate information on the quality and safety of local and imported rice samples acquired by the National Food Authority (NFA). Six rice samples, consisting of five local and one imported, were collected from different NFA warehouses and evaluated for grain quality and presence and concentration of pesticide residues and heavy metals. In terms of physical properties, the collected samples had high amounts of broken rice (32.1-52.6%) and chalky grains (12.7-25.6%). All the locally produced rice samples had long and intermediate-sized grains; while the imported sample from Pakistan possessed long and slender shape. The actual moisture content of rice samples ranged from 11.5% to 12.3%, which were slightly lower than the declared value (12-14%), but were within the ideal range (10-12%). All the local rice samples had high AC (22.4-24.6%) and intermediate GT (4.7 to 5.6). This high AC-intermediate GT combination tends to produce moderately tender cooked rice. The imported sample had intermediate AC (21.6%) and low GT (6.3), which is likely to have a moderately tender to hard cooked texture. The protein content of the samples (6.4-7.2%) were within normal range (6-9%). In terms of sensory properties, all raw samples had neither pleasant aroma nor off-odor, with different intensities of whiteness, gloss, translucency, and hardness. Their cooked forms were bland, slightly glossy, slightly tender, slightly smooth, and had no aroma, off-odor, and off-taste. The samples' organochlorine and organophosphorus pesticide concentrations were all below the maximum residue limit (MRL) for rice. The levels of the heavy metals arsenic, cadmium, lead, and mercury were likewise below the MRL. To evaluate the quality of staled cooked NFA rice, ten rice samples were evaluated for their microbial load and sensory index after storing at room and refrigerated temperatures. Refrigerated samples remained acceptable, with sensory index of 2.7-2.9 and lower microbial load than the samples stored at room temperature after 24h.

Development of NIRS Calibration Models for Rice Physicochemical Properties Evaluation

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Grain quality is one of the most important considerations in the rice breeding program. Grain quality evaluation includes the determination of rice milling potentials, physical attributes, physicochemical properties, cooking parameters, sensory characteristics, and nutritional quality. Starch physicochemical properties are among the deciding factors in selecting new rice breeds as these traits are the major contributors to the cooking and eating quality of rice. Amylose content (AC), protein content (PC), and gelatinization temperature (GT) are the major rice physicochemical properties. These parameters are measured in the laboratory by conventional chemical methods that are laborious, time-consuming, costly, and toxic to the analysts and the environment. Our group receives hundreds of samples for analysis. Therefore, there is a need to provide an alternative method that helps speed up the laboratory screening process. The near infrared reflectance spectroscopy (NIRS) offers a fast, simple, safe, and nondestructive analytical technique for food quality control. In this study, we calibrated the NIRS machine to predict the AC, MC, and PC of rice samples. Using modified partial least-squares regression, calibration models were developed from 580 milled rice and 470 flour samples representing a wide range of reference values. Results showed that calibration models built with flour samples were better than milled rice as indicated by the higher coefficient of determination, lower standard error of calibration, and standard error of prediction. Moreover, PC and MC calibration models for the flour sets were superior to the AC calibration model. The validation test showed that the developed models could predict the MC and PC values of unknown samples with 97% and 94% accuracy, respectively. However, the PC calibration model had a higher ratio of prediction to deviation value (2.4) relative to the MC calibration model (1.5), suggesting that the PC calibration model would likely predict the values accurately.

In conclusion, the NIRS machine can be used for rapid and nondestructive PC and MC screening of rice samples. Further refinements of the calibration models will be performed to improve the machine's prediction accuracy and reliability for routine screening of large rice samples. Adopting this instrument method would expedite the evaluation of rice's physicochemical properties because the machine can simultaneously analyze the PC and MC of around 80 to 100 samples per day. Also, the use and generation of toxic chemical waste from conventional protein analysis were eliminated.

PROJECT 5

Red Mold Rice as Natural Dietary Supplement: Market Studies

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The project assessed the marketability of red mold rice (RMR) as a functional ingredient or diet supplement. A survey among 30 local pharmaceutical companies revealed no existing RMR product being marketed in the country as a functional ingredient or supplement. A PhilRice-produced RMR-based supplement was developed using the previously optimized processing technology. The quality (total phenolic content, antioxidant activity, and mevinolin content) and safety (citrinin content) of the prototype were evaluated and compared with the 10 commercially available RMR-based supplements procured during key informant interviews and research discussions and through online shopping. The PhilRice-produced RMR supplement was either superior or comparable with the other RMR-based dietary supplements in terms of total phenolic content and antioxidant activity (1,1-diphenyl-2-picrylhydrazyl [DPPH], 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid [ABTS], and ferric reducing antioxidant power [FRAP]). It had the highest total phenolic content (14.43mg GAE/g) along with Red Yeast Rice from Kasetsart University in Thailand (14.47mg GAE/g). It was also one of the three best-performing supplements in terms of antioxidant activities. In terms of cholesterol-lowering property, the PhilRice-produced RMR supplement contained 5.0mg/kg mevinolin, which was significantly higher than the other commercially available RMR supplements in the market. It is also safe for human consumption as indicated by its significantly low citrinin level (0.6mg/kg), meeting the standards of Taiwan and the European Union (2.0mg/kg). Technology transfer of RMR can now be made with a manufacturing company as a coffee ingredient or diet supplement.

PROJECT 6

Improving Brown Rice Quality, Shelf Life, and Engineering Technologies

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Brown rice (BR), also known as "pinawa," is considered a whole grain food. This unpolished form of rice is nutritionally superior to milled or white rice because it contains higher amounts of fiber, protein, vitamins, minerals, and antioxidants. The enhancement of rice supply is another advantage of BR relative to polished rice because of its 10% higher milling recovery. The national BR campaign was officially launched in 2004 to celebrate the International Year of Rice, and the promotion of BR consumption has intensified since 2013 during the National Year of Rice. Due to the intensive campaign of the Department of Agriculture and the great efforts being made by researchers to improve the quality of BR, the awareness of consumers on its goodness and health benefits has increased. However, Filipino households still have very low consumption of BR because of poor eating quality, short shelf life, limited availability, and high price. Moreover, sustaining consumer interest and ensuring increased BR consumption among all Filipino populations are affected by several interplaying factors. Of these, ensuring good BR quality from the market to the table, varying consumer preferences, and changing dietary patterns and health practices are considered the most pressing.

Therefore, this project aimed to improve the quality and shelf life of BR and improve and pilot test BR engineering technologies. With this, the availability and accessibility of affordable BR in the market will be assured, thereby providing rice consumers a healthy, nutritious, and shelf-stable product.

A common concern with BR is its relatively poor eating quality, pertaining particularly to the hardness of its cooked rice. To address this, the project generated clusters based on AC and GT. The clustering was validated by determining the Instron hardness of the rice varieties in each cluster. The fats in BR are very susceptible to hydrolytic rancidity during storage. Thermal, chemical, moisture removal, and sterilization treatments have been investigated to extend the shelf life of BR. However, only a few of these methods were adopted on a commercial scale due to the change in the quality of the stabilized BR, which affects its consumer acceptability. Thus, this project screened different stabilization techniques, including both dry and wet heat treatments. Steam-heating followed by oven drying was the most effective in reducing the free fatty acid content and lipase activity of BR. This resulted in the longer shelf life of the stabilized BR.

The best packaging material and storage temperature were also investigated. Various methods of BR storage have also been tested to determine the temperature,

atmosphere, and kind of packaging that can prolong its shelf life but with limited success. In this project, the effects of hermetic storage technology (SACLOB) on the quality of BR, including rancidity, color, odor, eating quality, and nutritional characteristics, were determined. Moreover, the percent oxygen, moisture content, temperature, and relative humidity of BR in SACLOB were monitored during storage, and their effects on the quality of BR were also assessed.

Some studies suggest that BR can also help reduce the incidence of non-communicable diseases such as hypertension and diabetes. Thus, this project investigated the effects of BR consumption on the metabolic risk factors of obese/overweight Filipino adults to these diseases to validate this claim. A randomized controlled study was conducted wherein two groups of volunteer participants were formed depending on the kind of rice (brown or white rice) that they consumed during over six weeks of intervention. Their overweight/obesity parameters (body mass index, body fat percentage, visceral fat, waist circumference), blood pressure, fasting blood glucose, and lipid profile were monitored during pre- and post-intervention as well as follow-up sessions.

Lastly, this project also developed machines that can produce BR in the localities where the source of raw paddy is available. Traditionally, BR is produced using a mortar and pestle. Since this is no longer popular, BR produced by a dehulling machine is more commonly sold in supermarkets in the cities. As such, BR has limited accessibility, particularly in rural areas. The PhilRice-developed BR machines were composed of three models: portable, village-type, and retrofitted-type. Ten machines were deployed to cooperating users in Regions 2, 3, 6, and 9 as pilot units. Regular monitoring was conducted where the technical data and feedback were gathered. The results showed that the portable BR machine with a 4kg/h to 9kg/h capacity output had 58% to 69% milling recovery; the village-type model with the output of 73kg/h to 187kg/h had 58% to 67% recovery; while the retrofitted-type BR machine with 114kg/h to 116kg/h output had 67% to 77% milling recovery.