AMYLOSE CONTENT CALIBRATION MODEL FOR THE THREE TYPES OF SELECTED RICE GRAINS USING VISIBLE SHORTWAVE NEAR INFRARED SPECTROSCOPY

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DEDICATION

Dedicated with deepest love to:

“ My beloved family Mak, Romsah binti Saman,
Abah, Ibrahim bin Ahmad
My sisters and my brothers
Thanks for youradvice, guidance and love.

My cherish husband Mohd.Farid bin Husin,
Thank you so much for your time, support and patience.

My dearest friends for being there whenever I needed them.
Thank you for your cooperation and believed.”

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First of all, I would like thank to Allah for HIS firm hands in guiding me in the course of completing this thesis writing. It is by HIS grace and mercy that I am able to embark on the project within such a limited time. Alhamdulillah.

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Last but not least, to all my beloved family members who were always by my side to encourage, advice, comfort, cherish and support me during this entire project. This task had taught me a lot of lessons and knowledge which would be valuable to me in the future.

ABSTRACT

Amylose content is one of the main characteristics to measure the quality and texture of rice. This research aims to conduct a non-invasive measurementof amylose content in rice grains using a Visible-Shortwave Near-Infrared Spectroscopy (VIS-SWNIRS) through the combination of two methods: Principal Component Regression (PCR) and Artificial Neural Network (ANN). Three data sets of rice samples (spectral VIS-SWNIR and amylose content reference) from three types of rice (brown rice, basmati rice and white rice) that are available in the Malaysian market were used and processed separately. The effect of data shift in the reflection spectrum was eliminated using the zero, first and second order derivatives which were then combined with the zero, first and second order of the Savitzky-Golay filter. The data spectrum spread was reduced using Singular Value Decomposition (SVD). The PCR and ANN methods were applied with 65% of the data sets were used for training while the remaining 35% were used for testing. The research analysis results have found that the Root-Mean-Square-Error of Calibration (RMSEC),the correlation coefficient of calibration (r_c), the Root-Mean-Square-Error of Prediction (RMSEP), and the prediction correlation coefficient (r_p) of PCR for brown rice were 2.96, 0.44, 2.74, and 0.22 respectively. For basmati rice, the corresponding values were 1.93, 0.57, 1.98, and 0.40 while for white rice the values were 2.42, 0.73, 2.65, and 0.62. In the meantime, ANN analysis yields the values of 0.70, 0.99, 0.96, and 0.88 for brown rice, 0.24, 0.99, 0.31, and 0.99 for basmati rice and 1.03, 0.95, 1.05, and 0.93 for white rice. The results suggest that VIS-SWNIRS is suitable and has the potential to be used in the non-invasive assessment of amylose content in rice grains from three types of rice in the Malaysian market.

ABSTRAK

Amilosa merupakan salah satu ciri utama dalam menentukan kualiti dan tekstur beras. Kajian ini dijalankan untuk mengukur kandungan amilosa secara tidak invasif dalam bijirin beras dengan menggunakan alat *Visible-Shortwave Near Infrared Spectroscopy* (VIS-SWNIRS) melalui dua gabungan metod iaitu *Principal Component Regression* (PCR) dan Rangkaian Neural Tiruan (ANN). Tiga set data sampel beras (VIS-SWNIR spektra dan rujukan kandungan amilosa) daripada tiga jenis beras (beras perang, beras basmati dan beras putih) yang terdapat di pasaran Malaysia telah diguna dan diproses secara berasingan. Kesan peralihan data dalam spektrum pantulan telah dihapuskan dengan menggunakanterbitan perintah sifar, perintah pertama dan perintah kedua dan kemudian digabungkan dengan menggunakan penapis *Savitzky-Golay* perintah sifar, perintah pertama dan perintah kedua. Penyebaran data spektrum telah dikurangkan dengan menggunakan *Singular Value Decomposition* (SVD). Kaedah PCR dan ANN telah diuji menggunakan 65% daripada set data dan disahkan menggunakan 35% baki daripada set data berkenaan. Hasil analisis kajian mendapati Punca Min Kuasa Dua Ralat Penentukuran (RMSEC), pekali korelasi penentukuran (r_c), Punca Min Kuasa Dua Ralat Ramalan (RMSEP), pekali korelasi ramalan (r_p) PCR bagi beras perang adalah 2.96, 0.44, 2.74, 0.22 setiap satu. Manakala untuk beras basmati adalah 1.93, 0.57, 1.98, 0.40 dan beras putih pula adalah 2.42, 0.73, 2.65 dan 0.62. Analisis ujian ANN pula menunjukkan keputusan beras perang adalah 0.70, 0.99, 0.96 dan 0.88. Manakala untuk beras basmati adalah 0.24, 0.99, 0.31 dan 0.99, seterusnya bagi beras putih adalah 1.03, 0.95, 1.05 dan 0.93. Keputusan kajian mencadangkan bahawa penggunaan VIS-SWNIRS adalah sesuai dan berpotensi untuk digunakan dalam menilai kandungan amilosa terhadap bijirin beras secara tidak invasif dengan menggunakan tiga jenis beras dalam pasaran Malaysia.

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LIST OF ABBREVIATION

µm	-	Micrometer
A	-	Absorbance
AC	-	Amylose content
ANN	-	ANN
BERNAS	-	Padiberas NasionalBerhad
BP-ANN	-	Back-propagation Artificial Neural Network
CCP	-	Correct classification percent
C-H	-	Carbon-Hydrogen
cm^{-1}	-	One per Centimeter
C-O-H	-	Amylose content
CV	-	Cross-Validation
DM	-	Diabetes mellitus
DOM	-	Degree of milling
exp	-	Exponent
Far-IR	-	Far Infrared
FT-Raman	-	Fourier-Transform Raman
GI	-	Glycaemic Index
GNI	-	Gross National Income
HNO_3	-	Nitric acid
I_2	-	Iodine
ISO	-	International Standards Organization
KADA	-	Kemubu Agricultural Develoment Authority
KBB	-	Kilang Beras BERNAS
KI	-	Potassium Iodide
KOH	-	Pottasium Hydroxide
LMVC	-	Linear multivariate calibration

LOOCV	-	Leave-One-Out Cross-Validation
M_1 or M_2	-	Molarity
MARDI	-	Malaysian Agricultural Research and Development Institute
MIR	-	Middle Infrared
ml	-	Mililiter
mm	-	Milimeter
MSC	-	Multiplicative Scatter Correction
MSE or *mse*	-	Mean square error
N	-	Normal
NaOH	-	Natrium hydroxide
N-H	-	Nitrogen-Hydrogen
NIR	-	Near infrared reflectance
NIRS	-	Near-Infrared Spectroscopy
NIT	-	Near-Infrared Transmittance
nm	-	Nanometer
O-H	-	Hydroxyl bonds
OHP	-	Overhead Projector
PCR	-	Principal Component Regression
PCs	-	Principal components
PLSR	-	Partial Least Square Regression
PPK	-	Pertubuhan Peladang Kawasan
R	-	Reflectance
R&D	-	Research and development
R^2	-	Coefficient of determination
rc	-	Correlation Coefficent for calibration
RM	-	Ringgit Malaysia
RMSECV	-	Root mean square error cross-validation
RMSEP	-	Root mean square error
rp	-	Correlation Coefficent for prediction
S/N	-	Signal-to-noise ratio
SECV	-	Standard error cross validation
SG	-	Savitzky-Golay
S-H	-	Sulfur-Hydrogen or sulfhydryl group

SLC	-	Surface lipid content
SNV	-	Standard Normal Variate
SVD	-	Singular Value Decomposition
SWNIRS	-	Short Wavelength of Near Infrared Spectroscopy
T	-	Transmittance
TCA	-	Trichloroacetic acid
USA	-	United State
USDA	-	United State Department of Agriculture
UV	-	Ultraviolet
v_1 or v_2	-	Volume
VIS-SWNIRS	-	Visible Shortwave Near-Infrared Spectroscopy
X	-	X-axis
Y	-	Y-axis

LIST OF SYMBOLS

α	-	Alfa
%	-	Percent
/	-	or
r	-	Correlation coefficient
d	-	Diameter
h	-	height
~	-	Approaching
=	-	Equal
f	-	Force constant, 5×10^5dynes/cm
f	-	Model (model design) ot transfer function
y	-	Reference data
X	-	Spectral data (absorbance)
m	-	Cp2
m_1,m_2	-	Mass
k	-	Groups of data
n	-	Total number of sample
Io	-	Light intensity recorded by the reference photodiode
I	-	Light intensity recorded by the sample photodiode
^oC	-	Degree celcius
y	-	Measured amylose content or target
$\hat{y}$	-	The predicted of amylose content
$\bar{\hat{y}}$	-	The average value of $\hat{y}$
$\bar{y}$	-	The average value of y
A_λ	-	Wavelength-dependent absorbance
e_λ	-	wavelength dependent molar absorptivity

L	-	Effective path length of the light through the sample
C	-	Speed of light
c	-	Concentration of amylose content
A_c	-	Rectangular matrix
U	-	Normalized score matrix
S	-	Singular values
V	-	Loading matrix
p	-	The *i*-th predictor or input variable or nodes
PCv	-	Principal component for validation data set
A_v	-	Absorbance data for validation data set
X_0	-	Spectral data for training (calibration model)
y_0	-	Reference data for training (calibration model)
X_1	-	Spectral data for validation (calibration model)
y_1	-	Reference data for validation (calibration model)
X_p	-	Spectral data for testing (prediction model)
y_p	-	Reference data for testing (prediction model)
B	-	Least squares estimators
$\tilde{v}$	-	Frequency of vibration
x	-	The acquired spectrum
w	-	Weight
$\cong$	-	Approaching or equal to
$>$	-	Greater than
45^0	-	Fourty five degree
π	-	3.142

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Food quality and safety are one of the concerns that significantly affect governments, policy makers and communities around the world since it is closely related to public growth and health. However, among them staple food and its quality is the important issue as it provides vital production of energy required in human diet. Rice is the staple food in Asia, hence some Asian countries are world's major producers of rice such as Thailand, China, Vietnam, Myanmar, Cambodia and Indonesia [1].

Rice consists of starch; according to Copeland *et. al* [2], starch contributes 50% to 70% of the energy in human diet and it provides a direct source of glucose, which is an essential substrate in human brain and red blood cells in generating metabolic energy. In food and pharmaceutical industries, starch is used to influence or control characteristics such as texture, aesthetics, moisture, consistency and shelf stability [3].

However, the productivity of rice cultivation in Malaysia is quite low compared to other producing countries due to the lack of large scale farming as those done in these countries [1]. As illustrated in Figure 1.1, eight sub-sectors have generated domestic production with an approximate value of RM16.6 billion in 2009,

which covered 82% of the total contribution of agriculture to the country's Gross National Income (GNI) [1].

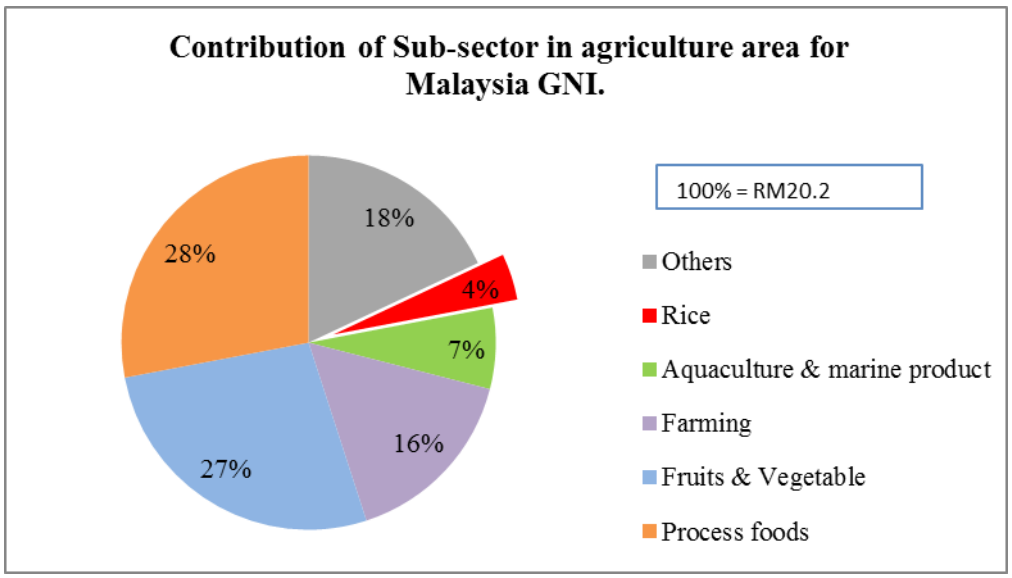

Figure 1.1 Contribution of agricultural sub-sectors to GNI, 2009 [1]

However, the contribution of rice farming to Malaysia's agriculture was only 4%, about RM0.8 billion of the total agricultural sub-sectors in GNI [1]. Besides that, Padiberas Nasional Berhad (BERNAS) reported that rice production in Malaysia in 2009 was only at 1.91 million tons, while the annual national consumption requirement of rice was 2.19 million tons [4]. This shows that the rice production in Malaysia was only sufficient for 70% for the total annual requirement.

In addition, the overall paddy production substantially decreased from 1.91 million tons in 2009 to 1.8 million tons in 2010 due to floods and diseases [1], [5]. These conditions lead to different qualities of rice obtained by BERNAS. However, as a committed company, BERNAS has put some effort to increase paddy production through the implementation of grading process in all BERNAS rice factory. One of the solution is by taking extra workers who are trained in paddy grading during harvesting seasons to make sure that all the rice reaped will be graded fairly and correctly [4].

Usually, rice quality is determined by its chemical, physical, cooking and also flavour characteristics [6]. There are many functional properties of rice that are closely related to physicochemical and other features, such as shape, size, degree of milling, percentage of head rice, gel consistency, gelatinisation temperature and amylose content [7]. These entire rice grain characteristics are the main interest area for the breeding program conducted by Malaysian Agricultural Research and Development Institute (MARDI) since 1992 to present. However, among all the characteristics, amylose content is the main trait for determining rice flavour and texture which will affect the properties of the final product [8]. Besides that, Rash and Meullenet [8] also stated that in the early stage of rice cultivation part, the differences in amylose content is used as a reliable data in the selection or deletion of genetic lines. Thus, it is necessary to elucidate the properties of three categories of rice grain (brown rice, basmati rice and white rice) in Malaysian market for their amylose content measurement.

In the past few years, a number of reports were published on the investigation of amylose content on the varieties of rice grain from Malaysian market by using visible shortwave near-infrared reflectance spectroscopy, especially for the rice that were harvested at several places in Malaysia such as Malaysian brown rice from Pahang (local rice) and Super Tempatan rice from Johor (local rice). Most references ([8]–[12]) showed that the varieties of rice grain quality were successfully assessed by using Near Infrared spectroscopy in the range of 1000nm to 2500nm. However, there is a lack of study on assessing the amylose content of rice grain in Malaysia by using visible and shortwave near the infrared spectroscopy in the range of 680 nm until 1000 nm.

Previously, [13] and [14] had examined the contribution and effect of physicochemical properties of amylose content towards human health. This is important since most of the Malaysian population consumes rice in daily and white rice which is the most commonly consumed has been associated with the development of Type 2 Diabetes Mellitus [6]. Therefore, this study puts forward a new approach in the agriculture field in Malaysia by exploring the use of simple,

environmentally safe [15], reliable and high speed [16] technique of Near-Infrared Spectroscopy (NIRS) in predicting the quality of rice grain.

Near Infrared analysis was first used by the United States' Department of Agriculture (USDA) in the mid-1960s in agriculture and food industries. Besides, it has been implemented for more than 25 years in pharmaceutical and chemical industries. It was also be able to make an improvement in the monitoring and control of industrial processes [17]. Therefore, the new initiatives and approaches highlighted in this study could benefit the Malaysian authorities by providing more information about amylose physicochemical properties that can offer a practical overview of the critical factors that are useful in implementing the Near-infrared Spectroscopy technique on rice grading in Malaysia. As a result, the study hopes to transform the paddy and rice industry in the country into a vibrant and progressive industry.

1.2 Problems Statement

In Malaysia, BERNAS is involved in the procurement and processing of paddy as well as the importation, warehousing, distribution and marketing of rice. Based on the rice and paddy control act of 1994, the grading process shall be carried out in BERNAS premises [18]; however, most of the rice production units faced a shortage of skilled workers to carry out the grading work to ensure that the rice were graded thoroughly [5]. As most of these units had to hire skilled workers to carry out the process of determining the quality of rice, the production cost increased since the workers have to be paid higher wages. Consequently, farmers are forced to bear the cost of the grading process for each rice crop in every season to as high as 20% [19][20].

Based on the supervision conducted by researchers at a BERNAS factory on 6 May 2012, it was found that the measurement tool used by BERNAS to measure

the quality of the rice is a Satake milling meter. This tool can only assess the quality of rice through physical features (degree of milling (DOM), transparency, chalkiness and whiteness) as the main criteria in determining the best quality of rice. As a result, the grading process of rice was not done fairly due to the DOM application [20]. Moreover, the conventional measurement technique using iodine colorimetric in determining the amylose quality of the rice is seen to have a number of weaknesses such as methods that are not environmentally friendly since it produces chemical waste, work procedure that takes a long time for each sample and requiring sample preparation process that is complicated ([8], [9], [11], [16], [21]).

Therefore, in order to achieve a better alternative in determining the quality of rice, this study employed a tool called the Visible Shortwave Near-Infrared Spectroscopy (VIS-SWNIRS) to measure the nutrient content in rice, especially for the determination of its amylose content. It is a simple and fast grading process technology. Besides, it offers non-destructive analysis even for intact solid or liquid samples and yields both chemical and physical information in development or production stage. Moreover, it is a chemical free technique, requiring no sample preparation and the quality of the sample can be assessed in just one scan [22].

According to [23], Malaysia is ranked ninth among the Asian countries with high diabetes mellitus (DM). The Obesity Prevention Council President, Jong Koi Chong said in The Star Press, in 2006 only 8.6% of adults in Malaysia had diabetes, compared with the most recent study done in 2013 which showed that 15% of adults were diabetic [24], [25]. The acute problem faced by the patients of type 2 diabetes are dehydration, electrolyte imbalance over short term, loss of vision (retinopathy), as well as kidney disease (nephropathy) over long term [14]. Therefore, awareness about the advantages of amylose content could be instilled through information provided on rice packaging. This communication would provide useful diet information, especially to those who suffer diabetic condition as well as to their family members. This is because, certain amount of amylose content is able to maintain blood glucose in human body and it is good for health.

Last but not least, the use of VIS-SWNIRS to measure the amylose content in rice has not been used by rice companies in Malaysia as yet. Therefore, this study aims to measure and predict the level of amylose content in rice by using VIS-SWNIRS. Besides, the instrument used in this study can add into the variety of alternative approaches that can be employed by rice manufacturers to detect and determine other quality parameters of rice grading using reliable and appropriate equipment. So, this study provides useful and new information in investigating rice quality based on the amylose content of the crop grown in Malaysia.

1.3 Objectives

The main objective of this study is to predict the amylose content in rice by using Visible Shortwave Near-Infrared Spectroscopy.

The specific objectives of this research are to:

i. evaluate the potential of Visible and Shortwave Near-Infrared Spectroscopy (VIS-SWNIRS) as a rapid method to estimate the level of amylose content (AC) on three categories of rice grain (brown rice, basmati rice and white rice) available in Malaysia market.

ii. analyse the performance of measured and predicted value of amylose content (AC) using the linear predictive model; Principal Component Regression (PCR).

iii. measure the correlation between destructive and non-destructive data using the non-linear predictive model, Artificial Neural Network (ANN).

1.4 Scope of Work

The scope of this project is to measure the quality of rice based on amylose content among three categories of rice available in Malaysia by using VIS-Shortwave Near-Infrared Spectroscopy (VIS-SWNIRS). The samples of the three categories of rice examined in this project are brown rice manufactured in Pahang, Maharani basmati rice imported from Pakistan and Super Tempatan (15%) rice manufactured in Johor respectively. The investigation identified the quality of rice based only on their amylose content. The instrument that was used in this experiment is the short wave Vis-Shortwave Near-Infrared Spectroscopy (VIS-SWNIRS) in the range of 680nm to 1000nm.

The linear and nonlinear models were designed to compare the data obtained from the destructive instrument (Iodine Colorimetric) and the non-destructive instruments (Visible Shortwave Near-Infrared Spectroscopy). Initially, the spectrum of brown rice, milled rice, basmati rice and its corresponding reference data were acquired via near infrared spectroscopy and Iodine colorimetric respectively. Next, for the linear model; the Principle Component Analysis was used to compress the dimension of input data before optimising the proposed predictive model. The optimum data component was then used as an input for the linear (Multiple Linear Regression) and a non-linear (Artificial Neural Network) models to obtain the value of the amylose content.

1.5 Significance of Research

1) Results from this research can broaden the research related to amylose content using rice samples (brown rice, basmati rice, and white rice) in the Malaysian market.

2) Apart from that, the research findings also diversify the use of Visible Shortwave Near-Infrared Spectroscopy (VIS-SWNIRS) to measure the amylose content using different rice grains.

3) The research adds to the existing reference materials on quality assessment of rice available in the Malaysian market for three types of rice (brown rice from Pahang, basmati rice from Pakistan, and white rice from Johor) using VIS-SWNIR Spectroscopy.

4) The analysis method on amylose reference data and VIS-SWNIR spectrum has been extended through the use of Principal Component Regression (PCR).

5) The process of assessing the correlation between amylose content and VIS-SWNIR has been improved by converting the linear regression method to nonlinear using Artificial Neural Network (ANN).

6) Additional reference about the use of spectrum pre-processing method such as the Savitzky-Golay derivative in more detail prior to the chemometric method is also provided.

7) The research has also set up a database for three rice types (brown rice from Pahang, basmati rice from Pakistan, and white rice from Johor) that is available in the Malaysian market.

1.6 Thesis Outline

The framework of the thesis is divided into six chapters. Chapter 1 contains the introduction of the research consisting of the background of the research, problem statement, objectives, scope and significance of study as well as the framework of the thesis.

Chapter 2 presents the literature review of the research which includes some information about amylose content, the advantages and history of the Near-Infrared Spectroscopy. Previous researches that relate with this work is also discussed in detail in this chapter.

Chapter 3 discusses the theoretical background of Near Infrared Spectroscopy, Principal Component Regression and Artificial Neural Network.

Chapter 4 elaborates the sample and chemical preparation as a part of the research methodology. Then, the experimental setup for each measurement is also explained in this chapter.

Chapter 5 focuses on the result and analysis of the measurements. The performance between linear and Non-linear model are compared in terms of the correlation coefficient and root mean square error. Besides that, the performance and quality of three categories of rice is also discussed.

Finally, Chapter 6 draws the conclusion for this study and suggests future research. In this chapter, the advantages and findings of the research are also concluded with some recommendation for future investigations.

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