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Determination of oil palm fresh fruit bunch ripeness—Based on flavonoids and anthocyanin content

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ABSTRACT

Non-destructive and real-time oil palm fresh fruit bunch (FFB) grading systems are of major exploratory concern for researchers in the oil palm industry. The objective is to reduce time, labour, costs, and most importantly, to increase the oil extraction rate, in order to achieve a good quality of palm oil at a more acceptable price. This research investigates the potential of flavonoids and anthocyanins as a predictor to classify the degree of oil palm FFB ripeness. This paper also discusses the relationship between these predictors and the ripeness categories period. One hundred and eighty oil palm FFB samples were collected from a private plantation in Malaysia, according to three maturity categories i.e., ripe, under-ripe, and over-ripe. Each sample was randomly scanned 10 times, both front and back using a hand-held Multiplex®3 multi-parameter fluorescence sensor. The results show that flavonoid and anthocyanin content decreased from immature to over mature oil palm FFBs. Overall, the relationship using Pearson's correlation between flavonoids and anthocyanins was $r^2 = 0.84$ and the most outstanding relationship accuracy was at the over-ripe stage, at 90%. Statistical analysis using analysis of variance (ANOVA) and pair-wise testing proved that both predictors gave significance difference between under-ripe, ripe, and over-ripe maturity categories. This shows that both predictors can be good indicators to classify oil palm FFB. Classification analysis was performed by using both predictors together and separately through several methods. The highest overall classification accuracy was 87.7% using a Stochastic Gradient Boosting Trees model and with both predictors. The other classification methods used either independent or both predictors together and gave various results ranging from 50 to 85% accuracy. This research proves that flavonoids and anthocyanins can be used as predictors of oil palm maturity classification.

1. Introduction

Malaysia produces an average of 19.2 tonnes of oil palm Fresh Fruit Bunches (FFB) per hectare (MPOB, 2010). The total oil palm planted area of Malaysia increased by 4.5% to 4.69 million hectares in 2009 (Wahid, 2010). Malaysia currently accounts for 39% of world palm oil production and 44% of worldwide exports (MPOC, 2009). Small holders account for 40% of plantation ownership, with many being allocated by the Government for settlement plots (Basiron, 2007; Teoh, 2010). Malaysia has actively planted oil palms commercially since the 1960s. A great deal of research has been carried out on oil palms in relation to increased yield, management, cloning technology, planting materials, specific fertilizers, disease protection and detection, processing, benefits, and mores. It consists of mainstream and downstream industry (Hai, 2002). The implementation of new technology, to improvise the current system in the oil palm sector, is necessary to sustain the growing global need for oils and fats.

Currently, most research on automated oil palm FFB grading systems is based on the surface colour of the fruitlets. 'Fruitlet' is defined as a single or individual fruit from an oil palm FFB (Fig. 1). Colour-based machine vision developed by researchers (Abdullah et al., 2001, 2002) has been proven able to differentiate fruitlets into correct ripeness categories, with 90% classification accuracy. The relationship of the fruitlets surface colour and oil content also gives a positive correlation (Balasundram et al., 2006; Choong et al., 2006; Shariff et al., 2004). Further research on the relationship between oil content and oil palm FFB has also given positive results (Hudzari et al., 2010; Tan et al., 2010). The Malaysian Palm Oil Board (MPOB) has suggested that colour metering could be used to determine ripeness, since there was no significant difference in fruit ripeness determination between using the colour meter and the human grader (Idris et al., 2003). When using the colour meter, the operator first needs to slice the fruitlets in order to expose a flat surface

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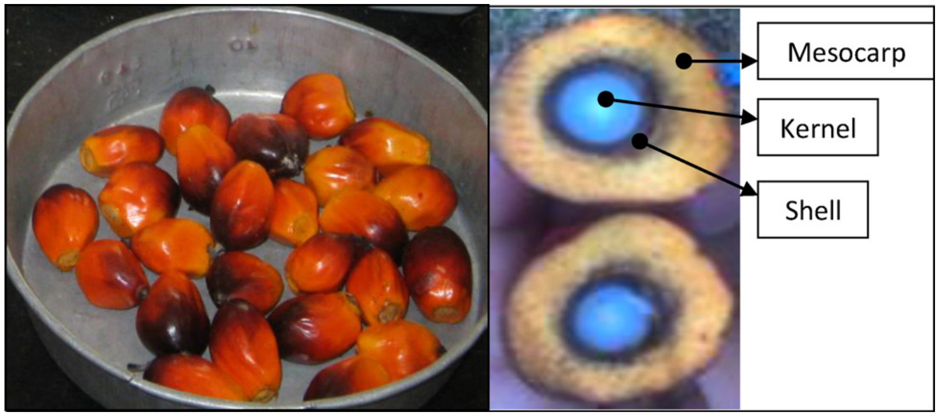

Fig. 1. Oil palm fruitlets and its characteristics.

of the mesocarp. The colour meter is then used to sense the mesocarp's colour. Due to time constraints, the colour meter is not well suited for an industrial application of oil palm FFB determination.

Now, the industries needs and the researcher's challenge is to focus on differentiating oil palm FFB into ripeness categories, based on bunches and not on each individual fruitlet. Many researchers have already developed optical properties and a program system that uses colour intensity, segmentation, and various colour spaces to differentiate oil palm FFB into specific ripeness categories (Alfatni et al., 2008; Ishak et al., 2000). The machine vision system is able to use any available colour space, either RGB, YCMK, HIS, CIElab, and many more (Alfatni et al., 2011; Cheng et al., 2001; Zheng et al., 2006). The results show that the accuracy of classification varies from 80 to 95%, which is 20% more consistent than a human grader (Abdullah et al., 2004). The model developed under laboratory conditions gave high accuracy; however, when tested in the field, the accuracy can reduce by more than 50% (Jamil et al., 2009). This may happen due to inconsistent light sources, humidity, temperature, oil palm FFB condition, among others (Ishak and Razali, 2010; Razali et al., 2008).

A hyperspectral imaging method has been deployed by developing a non-destructive technique for measuring the quality of an oil palm FFB (Osama et al., 2010; Phorntipha et al., 2009). Researchers found that the visible range between 600 and 1000 nm were the best wavelengths to discriminate oil palm FFB into their ripeness categories. This finding is based on the surface colour of the oil palm FFB. In reality, the physical characteristics of the oil palm FFB are subject to damage, both during the harvesting and transportation processes. There is a possibility of lipolytic enzyme or enzymes that are present in ripe palm fruits, to be released when the fruit is damaged (Chong and Sambanthamurthi, 1993). This enzyme changes the physical characteristics of the oil palm FFB and can affect its colour properties. Researchers also used moisture content extraction, by using tomographic radar imaging and a rectangular dielectric waveguide as a predictor, to detect the ripeness stage of the oil palm FFB (Abdullah et al., 2004; Mokhtar, 2004). Advanced methods using MRI and bulk NMR techniques have been employed to monitor the development of oil palm fruits and to follow the ripening process by measuring the progressive changes in the spin–spin relaxation times (T2-values) of the protons of the water and lipids (Sharifudin et al., 2010).

Manual grading of oil palm FFB into ripeness categories is a very difficult and tedious task, even for an expert grader. Therefore, there is a need for technology to assist humans in oil palm ripeness inspection. In this paper, we introduce a method of using flavonoids and anthocyanin content to predict and classify oil palm FFB into different categories of ripeness. Both are known as phenolic content inside the plant cell system (Cerovic et al., 1999). The role of this phenolic content is well known as a resistance of plants to insect attack, the attraction of animals for pollination, seed dispersal, and important functions in cognitive decline and neural dysfunction (Castañeda-Ovando et al., 2009; Diabaté et al., 2010; Kong et al., 2003). We measured the presence of flavonoids and anthocyanin within the epidermis using a fluorescence technique, which has been widely used in the grape industry, and gave promising and positive results to (Agati et al., 2011; Burlingame, 2008; Cerovic et al., 2008; Lenk et al., 2007; Louis et al., 2009; Meyer et al., 2009). Fluorescence is the latest sensing technique used for leave monitoring and fruit quality and maturity assessment (Kolb and Pfündel, 2005). Researchers have also used the non-contact and non-destructive nature of this method to identify spatial variation in plantation regions (Debuisson et al., 2010).

The objective of this study was to assess the potential of the fluorescence sensor to detect the ripeness of oil palm FFB quality; specifically skin anthocyanin and flavonol content. Flavonoids are present in the epidermis and they absorb UV radiation at the same time screen the mesophyll. Chlorophyll, from the mesophyll, emits near-infrared fluorescence measurable on the plant leaf and fruit. While anthocyanins become present in the epidermis by absorbing the green light and also screen the mesophyll (Goulas et al., 2004).

This is the first ever analysis of the oil palm FFB quality attributes obtained by this sensor. In this study, we identify the behaviour of anthocyanin and flavonoids in oil palm FFB in different ripeness categories. The positive outcome of this research shows their potential as predictors for oil palm FFB ripeness. Only three critical categories of oil palm FFB were used in this study, under-ripe, ripe, and overripe. Fig. 2 shows the surface colour and condition of the oil palm FFB in different ripeness categories.

2. Materials and methods

All data collection was performed at an oil palm mill in Peninsular Malaysia. One hundred and eighty samples were collected according to the three categories of ripeness.

2.1. Grading standard

The Malaysian Palm Oil Board (MPOB) published an oil palm FFB grading standards guideline (MPOB, 2006). This guideline was used in this research. All players involved in the oil palm sector must follow this guideline to avoid misclassification of oil palm ripeness category. Each category has different characteristics, which are summarized in Table 1. One of the most important factors is the mesocarp colour. If the colour is orange, it can be classified as ripe; otherwise, if the colour is yellow or yellowish/orange, it can be either unripe or under-ripe (Abdul et al., 2009). The number of

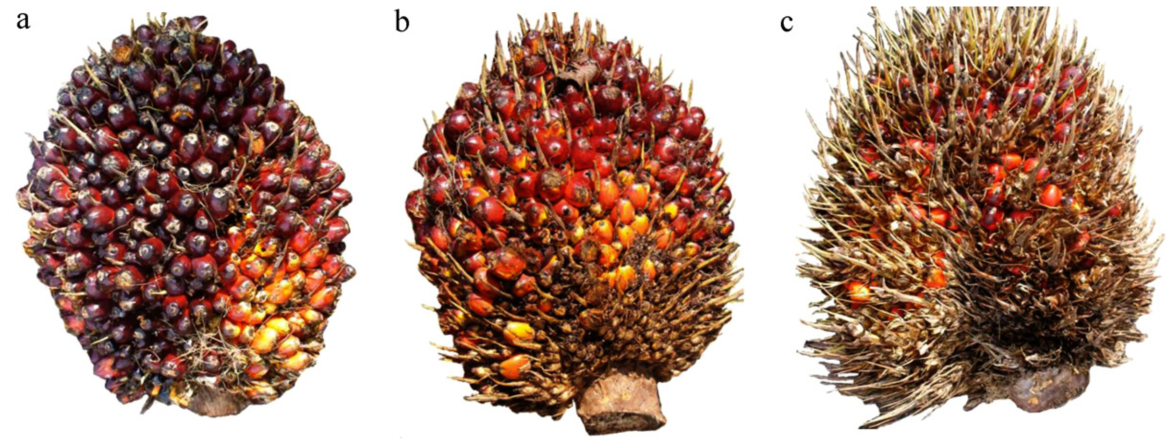

Fig. 2. Surface colour and condition of different ripeness's of oil palm FFB; (a) under-ripe; (b) ripe; and (c) over-ripe.

Table 1
Oil palm FFB ripeness categories based on total number of empty sockets and mesocarp colour.

Total number of empty fruitlet sockets	Mesocarp colour		
	Yellow	Yellowish/orange	Orange
0	Unripe	Unripe	Ripe
0–10	Unripe	Under-ripe	Ripe
>10	Unripe	Ripe	Ripe

empty fruitlet sockets can help to increase classification accuracy. Researchers and plantations have also defined oil palm FFB categories based on the number of fruitlets detached from a bunch. If more than 10% of oil palm fruits from the total fruitlets of one bunch are detached, it will be categorized as ripe (Choong et al., 2006) and other descriptions are shown in Table 2. In this study, we followed all guidelines, including the Sime Darby Palm Oil Mill standard (SDPB, 2004) and advice given from experienced oil palm FFB mill graders.

2.2. Instrumentation

The determination of flavonoids and anthocyanin contents was performed using a hand-held multi-parametric fluorescence sensor, controlled by a computer with four light sources (excitation Light Emitting Diode (LED)) and three photodiodes for detecting fluorescence emission. This consists of three LED-matrix light sources (Shark series, Opto Technologies, Wheeling, IL, USA): 375 nm UV-A (UV), 530 nm Green (G), and 630 nm Red (R), pulsed at 3.3 kHz (20 μs per flash). There were three synchronized detectors for fluorescence recording: Blue–Green (BGF), Red (RF) and Far-Red (FRF), based on three 20 mm × 20 mm silicone photodiodes (PDB-C618, Photonic Detectors, Simi Valley, CA, USA) protected by a 447WB60 (Semrock, Rochester NY, USA), 678WB22 and 750WB65 (Intor, Socorro, NM, USA) interference filters (Cerovic et al., 2008). This sensor is based on non-contact of leaf and fruit auto fluorescence measurements (Force-A, Force, 2009). The abilities of this sensor are that it can be used in the field, in daylight, or indoors, its speed (milliseconds) and its ability to analyse either the whole bunch or individual fruits (Agati et al., 2008, Cerovic et al., 2009).

Table 2
Classification of oil palm FFB ripeness based on detached fruitlets.

Categories	Descriptions
Ripe	10%–50% of fruits detached from bunch
Over-ripe	50%–90% of fruits detached from bunch
Under-ripe	1–9 fruits detached from bunch

The parameter used focused on phenolic maturity content, which is flavonoids (FLAV) and anthocyanin (ANTH). The parameter FLAV is represented by a log ratio from emission of far-red fluorescence with a red excitation (FRF_R) divided to the far-red fluorescence with UV excitation (FRF_UV) defined as:

$$\text{FLAV} = \log(\text{FER\_RUV}) = \log(\text{FRF\_R}/\text{FRF\_UV}), \quad (1)$$

where, FER_RUV is a fluorescence excitation ratio with red and UV excitation.

The ANTH parameter is a log ratio from emission of far-red fluorescence with a red excitation (FRF_R) divided by the far-red fluorescence with green excitation (FRF_G) given by:

$$\text{ANTH} = \log(\text{FER\_RG}) = \log(\text{FRF\_R}/\text{FRF\_G}), \quad (2)$$

where, FER_RG is a fluorescence excitation ratio with red and green excitation.

2.3. Samples

The fresh oil palm FFB samples were sourced from local plantations, which were sent to the palm oil mill within 24 h of harvesting. For each category of oil palm FFB, 60 samples were collected.

2.4. Sample preparation

All samples were tagged and arranged according to their categories. The selection of samples was performed by following MPOB guidelines, with advice from a certified and experienced palm oil mill grader. Each sample was randomly scanned 10 times, both front and back, using the fluorescence sensor.

2.5. Statistical analysis

All statistical analyses were performed using SPSS 16.0 for windows, SPSS Inc., Chicago. The relationship between flavonoids and anthocyanin was carried out using Pearson's correlation method (Fah and Hoon, 2009). An overall analysis-of-variance (ANOVA) test was conducted to assess whether the means of the dependent variable were significantly different among groups, or not (Green and Salkind, 2000). This analysis can be stated to reflect mean differences or relationships between variables. ANOVA was also used to identify whether at least two groups were significantly different mean among unripe, under-ripe, ripe, and over-ripe groups. As recommended, Linear Significant Difference (LSD) was used when dealing with only three groups (Welkowitz et al., 2006).

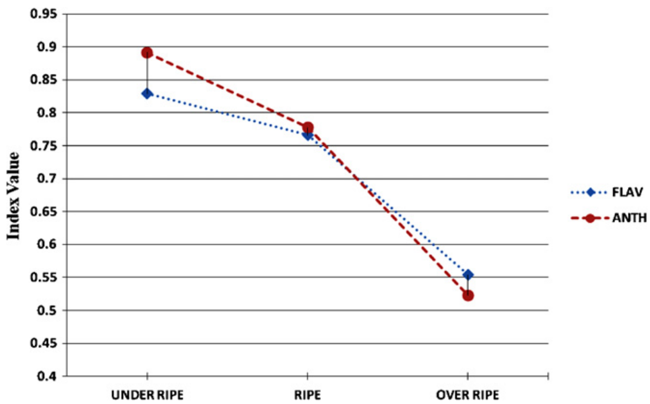

Fig. 3. Means of anthocyanin and flavonoid index values based on different ripeness categories of oil palm FFB.

2.6. Classification analysis

Several classification methods were performed in this study to identify the highest accuracy by using flavonoids, anthocyanin, or its interaction as predictors. This classification analysis was conducted using STATISTICA 8.0 (StatSoft, 2007). The technical details used for classification, as discussed in this paper, can be gathered from previous studies (Friedman et al., 2000; Friedman, 1991, 1999a, 1999b, 2001; Hastie et al., 2009; Hill and Lewicki, 2007; Nisbert et al., 2009).

2.6.1. Stochastic gradient boosting trees

Stochastic gradient boosting tree is an evolving method from the application of boosting methods to regression trees. The technique was expanded from regression on the prediction of the continuous dependent variable into the classification problem (Hill and Lewicki, 2007). This method and its algorithm are described in detail by (Friedman, 1999a, 1999b).

2.6.2. Interactive tree classification and regression tree (C&RT)

The purpose of C&RT, using tree-building algorithms, is to determine an accurate prediction or classification tree by a set of if-then logical (split) conditions (Hill and Lewicki, 2007). Historically, this algorithm was introduced by Breiman et al. (1984) and was developed for identifying high-risk patients at the University of California, San Diego's Medical Centre (Nisbert et al., 2009). The method becomes applicable in multi-disciplinary areas. In this study, Gini score is used as a default setting, which is based on the relative frequency of sub-ranges in the predictor variables, and was used to select the measure of "impurity" to use in evaluating candidate split points (Ripley, 1996).

2.6.3. General stepwise linear discriminant analysis

General discriminant analysis (GDA) relates the methods of the general linear model to the discriminant function analysis problem. A general overview of discriminant function analysis, and the traditional methods for fitting linear models with categorical dependent variables and continuous predictors, is provided in the context of general discriminant analysis. This discriminant function analysis is limited to simple and stepwise analyses with a single degree of freedom of continuous predictors. The advantage of this method is that the user can specify complex models for the set of predictor variables. Further detailed explanations and applications can be found through (Abdullah et al., 2001; Hill and Lewicki, 2007; Nisbert et al., 2009).

Table 3
Pearson's correlation between anthocyanin and flavonoids; (a) overall samples; (b) under-ripe category; (c) ripe category; (d) over-ripe category.

Correlations			
		ANTH	FLAV
(a)			
ANTH	Pearson correlation	1	.84**
	Sig. (two-tailed)		.00
	N	180	180
FLAV	Pearson correlation	.84**	1
	Sig. (two-tailed)	.00	
	N	180	180
(b)			
ANTH	Pearson correlation	1	.62**
	Sig. (two-tailed)		.00
	N	60	60
FLAV	Pearson correlation	.62**	1
	Sig. (two-tailed)	.00	
	N	60	60
			(*c*)
ANTH	Pearson correlation	1	.43**
	Sig. (two-tailed)		.00
	N	60	60
FLAV	Pearson correlation	.43**	1
	Sig. (two-tailed)	.00	
	N	60	60
(d)			
ANTH	Pearson correlation	1	.90**
	Sig. (two-tailed)		.00
	N	60	60
FLAV	Pearson correlation	.90**	1
	Sig. (two-tailed)	.00	
	N	60	60

** Correlation is significant at the 0.01 level (two-tailed).

2.6.4. MARSplines

The STATISTICA Multivariate Adaptive Regression Spines (MARSplines) module is a generalization technique popularized by Friedman (1991) for solving regression and classification type problems. Its purpose is to predict the value of a set of dependent or outcome variables from a set of independent or predictor variables (Hill and Lewicki, 2007). MARSplines can handle both categorical and continuous variables (whether response or predictors). In the case of categorical responses, MARSplines will treat the problem as a classification problem. This method has non-parametric procedures that make no assumption about the underlying functional relationship between dependent and independent variables (Nisbert et al., 2009). Recently, this technique has been widely used in the area of data mining, because it does not assume or impose any particular type or class of relationship between the predictor variables and the dependent (outcome) variable of interest. Detailed information about this technique and the algorithm is stated by Friedman (1991) and Hill and Lewicki (2007).

2.6.5. STATISTICA automated neural network

A neural network is an easy to use, powerful tool, because it learns from examples. The basic idea comes from a functional

Table 4
Homogeneity test (Levene's test) results for anthocyanin.

Levene's test of equality of error variances[a]			
Dependent variable:ANTH			
F	df1	df2	Sig.
1.401	2	177	.249

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
[a] Design: Intercept + CATEGORIES.

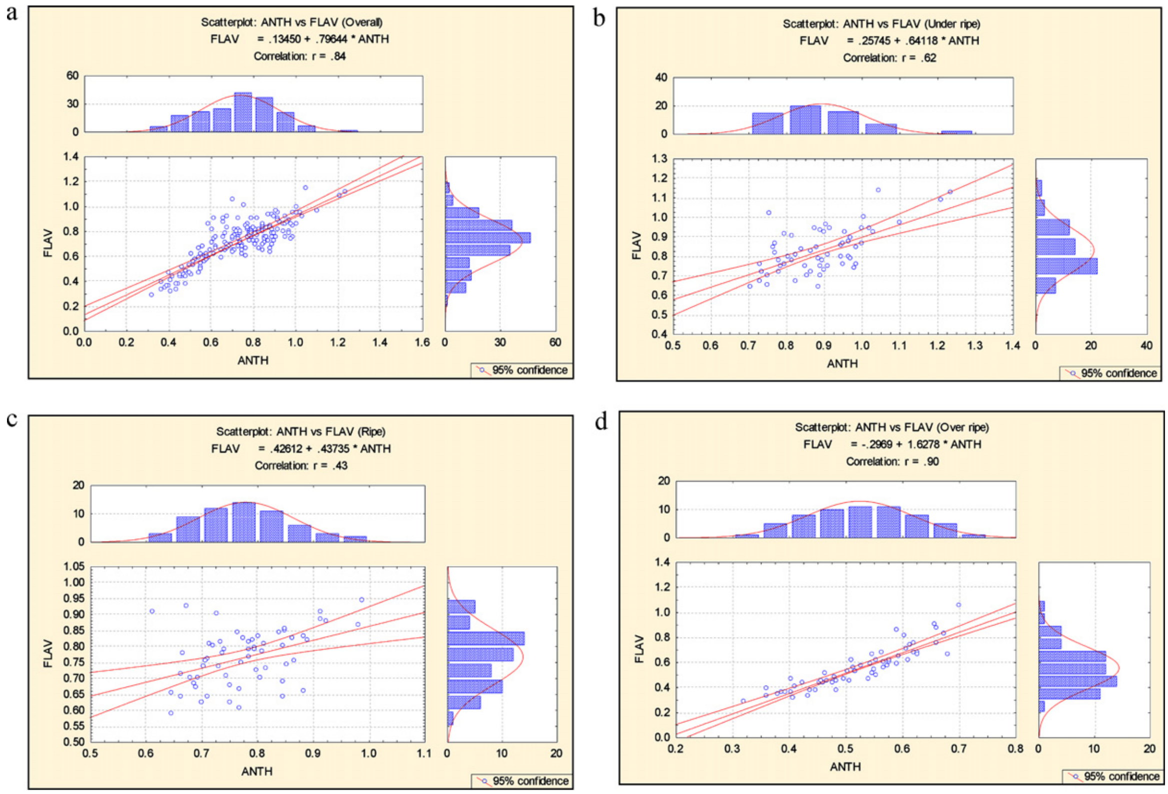

Fig. 4. Relationship between anthocyanin and flavonoids; (a) overall samples; (b) under-ripe category; (c) ripe category; (d) over-ripe category.

human neuron structure. All of the layers, including hidden layers, are connected to the preceding layer (Hill and Lewicki, 2007). Expanded details are explained by Nisbert et al. (2009) on how this tool runs to produce an algorithm and method for selecting the best classification accuracy.

2.6.6. *Support vector machine (SVM)*

This method performs classification problems by developing non-linear decision boundaries. Due to the nature of the feature space in which these boundaries are found, SVM can exhibit a large degree of flexibility in handling the classification tasks of varied complexities. For the classification analysis, STATISTICA SVM supports two types of Support Vector models, with a variety of kernels as a basis of function expansions. In this study, SVM Type 1 was used as standard, for comparing the results from different predictors. Detailed information on this technique has been discussed in previous research (Grace, 2006; Hill and Lewicki, 2007; Javier et al., 2006; Moore, 2003; Nisbert et al., 2009).

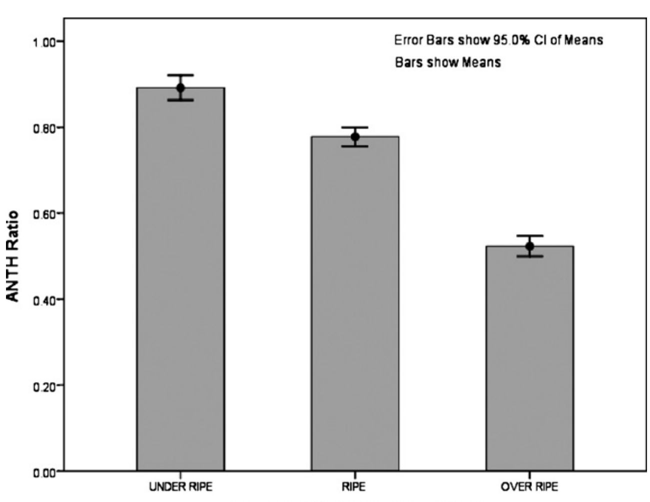

Fig. 5. The error bar chart for ANTH ratio with different oil palm FFB ripeness categories.

2.6.7. *Naïve Bayes classifiers*

An early Bayesian method was formulated to perform classification tasks. The first assumption is that the independent variables are statistically independent. A Naïve Bayes model is an effective classification tool that is easy to use and interpret. Naïve Bayes is suitable when the dimensionality of the independent space is high (Nisbert et al., 2009). For this reason, Naïve Bayes can often go one better than other more sophisticated classification methods. STATISTICA Naïve Bayes provides a variety of methods for modelling the conditional distributions of inputs, including normal, lognormal, gamma, and Poisson (Hill and Lewicki, 2007). For the purpose of study, all settings were at their default for all analyses.

2.6.8. *K-nearest neighbours (KNN)*

The specialty of K-nearest Neighbours is a memory-based method, which is differs from other statistical methods. This technique does not require a training phase. It is also known as the Prototype Method. The basic perceptive idea is that any close

Table 5
Welch and Brown–Forsythe *F*s results for anthocyanin data.

Robust tests of equality of means				
ANTH	Statistic[a]	df1	df2	Sig.
Welch	220.991	2	116.649	.000
Brown–Forsythe	228.709	2	167.615	.000

[a] Asymptotically *F* distributed.

Table 6
Results of between-subjects effect for anthocyanin data.

Tests of between-subjects effects						
Dependent variable: ANTH						
Source	Type III Sum of Squares	df	Mean square	F	Sig.	Partial η^2
Corrected model	4.281[a]	2	2.140	228.709	.000	.721
Intercept	96.045	1	96.045	1.026E4	.000	.983
CATEGORIES	4.281	2	2.140	228.709	.000	.721
Error	1.656	177	.009			
Total	101.982	180				
Corrected total	5.937	179				

[a] r^2 = .721 (Adjusted R^2 = .718).

Table 7
ANOVA results for anthocyanin data.

ANTH	Sum of squares	df	ANOVA		
			Mean square	F	Sig.
Between groups	4.281	2	2.140	228.709	.000
Within groups	1.656	177	.009		
Total	5.937	179			

Table 8
Descriptive statistics of anthocyanin data.

	Descriptive statistics			
Dependent variable:ANTH				
Categories	Mean	Std. deviation	Variance	N
Under-ripe	.8915	.11133	.01239	60
Ripe	.7774	.08471	.00718	60
Over-ripe	.5226	.09222	.00850	60
Total	.7305	.18212	.02807	180

objects are more likely to be in the same category. The prediction functions of KNN are based on a set of prototype examples, used to predict new data based on the majority vote over a set of K-nearest prototypes (Hill and Lewicki, 2007; Nisbert et al., 2009).

2.7. Results and discussions

The results obtained are discussed as follows; anthocyanin and flavonoids behaviour in the oil palm FFB ripeness process; relationship between anthocyanin and flavonoids; statistical analysis; and classification analysis.

2.8. Anthocyanin and flavonoids behaviour in the FFB ripeness process

Anthocyanin and flavonoid contents were determined by the index value of the fluorescence sensor. These results are plotted in Fig. 3, where it can be seen that when the bunch is at the under-ripe stage, the highest index values are given. This means that the fluorescence sensors can easily sense the presence of both contents at this stage. Then, the index value drops of slightly as the oil palm FFB changes to ripe and finally, over-ripe. For the anthocyanin, the index value was 0.89 for under-ripe, which then reduced to 0.77 and 0.52 for ripe and over-ripe, respectively. Meanwhile, the index values for the flavonoids at the unripe stage begin at 0.83 and then decrease to 0.78 and 0.52 for the ripe and over-ripe stages, respectively. This explains the nature of any living material (Dey et al., 1997), and these results show that the anthocyanin and flavonoid contents decreased with time.

The biological activities for phenolic maturity are more active at the immature stage and reduce or even stop at the over-ripe phase.

Table 9
The results of post hoc pair-wise comparisons for anthocyanin data.

Multiple comparisons							
Dependent variable: ANTH							
	(I) Categories	(J) Categories	Mean difference (I–J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
LSD	Under-ripe	Ripe	.1141*	.01766	.000	.0792	.1490
		Over-ripe	.3689*	.01766	.000	.3340	.4038
	Ripe	Under-ripe	−.1141*	.01766	.000	−.1490	−.0792
		Over-ripe	.2548*	.01766	.000	.2199	.2897
	Over-ripe	Under-ripe	−.3689*	.01766	.000	−.4038	−.3340
		Ripe	−.2548*	.01766	.000	−.2897	−.2199
Games-Howell	Under-ripe	Ripe	.1141*	.01806	.000	.0712	.1570
		Over-ripe	.3689*	.01866	.000	.3246	.4132
	Ripe	Under-ripe	−.1141*	.01806	.000	−.1570	−.0712
		Over-ripe	.2548*	.01617	.000	.2164	.2932
	Over-ripe	Under-ripe	−.3689*	.01866	.000	−.4132	−.3246
		Ripe	−.2548*	.01617	.000	−.2932	−.2164

Based on observed means. The error term is mean square (error) = .009.

* The mean difference is significant at the .05 level.

Table 10
95% confidence intervals of pair-wise differences in mean changes in oil palm FFB categories.

Oil palm FFB categories	*M*	*SD*	Under-ripe	Ripe
Under-ripe	.89	.11		
Ripe	.78	.09	.0792–.1490	
Over-ripe	.52	.09	.3340–.4038	.2199–.2897

Table 11
Homogeneity test (Levene's test) results for flavonoids.

Levene's test of equality of error variances[a]			
Dependent variable:FLAV			
F	df1	df2	Sig.
11.084	2	177	.000

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
[a] Design: Intercept + CATEGORIES.

Table 12
ANOVA results for flavonoid data.

FLAV	Sum of squares	df	Mean square	*F*	Sig.
Between groups	2.498	2	1.249	77.438	.000
Within groups	2.855	177	.016		
Total	5.353	179			

Table 13
Welch and Brown–Forsythe *F*s results for flavonoid data.

Robust tests of equality of means				
FLAV	Statistic[a]	df1	df2	Sig.
Welch	56.603	2	111.455	.000
Brown–Forsythe	77.438	2	138.851	.000

[a] Asymptotically *F* distributed.

The skin colour of the oil palm FFB varies from one to another. It is from light blue/green to orange/red in colour, the depth of colour depending on the amount of carotenoids present (Hadi et al., 2009). Like an apple, date, or grape, the red colour of oil palm FFB is due to the anthocyanin pigment (Al-Farsi et al., 2005; Honda et al., 2002; Li et al., 2004; Neo et al., 2010). When the oil palm FFB is in its maturing stage, the colour changes from green to orange with brown or black cheek colours (Esnan et al., 2004). The index value results confirm that the red coloured area decreasing means that the anthocyanin pigment has vanished during the oil palm FFB maturing process. Detailed information of anthocyanin behaviour and functionality has already been discussed elsewhere (Kong et al., 2003; Li et al., 2004), and suggests that cell length and thickness of the epidermis in oil palm fruitlets, like an apples is related to the anthocyanin increase in skin. Flavonoids occur in the form of glycosides in plants (Yang et al., 2009). The results illustrate that glycosides decrease, and the best reason for this is that the oil palm FFB does not need them anymore, after it has reached the highest level of maturity.

Table 15
Descriptive statistics of flavonoid data.

	Descriptive statistics			
Dependent variable: LAV				
CATEGORIES	Mean	Std. deviation	Variance	*N*
Under-ripe	.8290	.11489	.01320	60
Ripe	.7661	.08692	.00756	60
Over-ripe	.5537	.16623	.02763	60
Total	.7163	.17293	.04839	180

2.9. Relationship between flavonoids and anthocyanins

The overall value of Pearson's correlation coefficient, $r^2 = 0.84$ shows (Fig. 4a; Table 3a) that there is a strong positive relationship between anthocyanin and flavonoid content, during the maturity process. If the anthocyanin content inside the oil palm FFB increases, the flavonol content will also increase. Conversely, decreasing anthocyanin content will decrease flavonoid content. This strong relationship is apparent in the over-ripe category, where the value of Pearson's correlation coefficient, $r^2 = 0.90$ (Fig. 4d; Table 3d). It is subtly related that both phenolic contents disappear when oil palm FFB is over-ripe. Under-ripe and ripe oil palm FFB categories gave a weak positive relationship with, $r^2 = 0.60$ (Fig. 4b; Table 3b) and $r^2 = 0.43$ (Fig. 4c; Table 3c). At both stages, the contents of those phenolics was weak due to the maturing process involving various factors based on the needs and functions of the phenolic itself and environment factors. These factors could be seasonal, temperature, humidity, fertilizer, oil palm management, and others.

2.10. Statistical analysis

A one-way analysis of variance was conducted to evaluate two types of relationship and their mean differences among the three oil palm FFB ripeness categories. The first relationship evaluated was between oil palm FFB categories and anthocyanin content. The second relationship was between oil palm FFB categories and flavonoid content.

2.10.1. Statistical analysis for anthocyanin

The error bar chart of anthocyanin data (Fig. 5) illustrates the magnitude of the mean for each category of the oil palm FFB in each category; the 'I' shapes show the confidence interval of these means. The error bar shows that there was significance difference of variance across the samples. A one-way analysis of variance (ANOVA) was conducted to evaluate the relationship between anthocyanin content and the oil palm FFB ripeness categories. The

Table 14
Results of between-subjects effect for flavonoid data.

Tests of between-subjects effects						
Dependent variable:FLAV						
Source	Type III sum of squares	df	Mean square	*F*	Sig.	Partial η^2
Corrected model	2.498[a]	2	1.249	77.438	.000	.467
Intercept	92.350	1	92.350	5.726E3	.000	.970
Categories	2.498	2	1.249	77.438	.000	.467
Error	2.855	177	.016			
Total	97.703	180				
Corrected total	5.353	179				

[a] $R^2 = .467$ (Adjusted $R^2 = .461$).

Table 16
The results of post hoc pair-wise comparisons for anthocyanin data.

Multiple comparisons							
Dependent variable:FLAV							
	(I) Categories	(J) Categories	Mean difference (I–J)	Std. error	Sig.	95% Confidence interval	
						Lower bound	Upper bound
LSD	Under-ripe	Ripe	.0629*	.02319	.007	.0172	.1087
		Over-ripe	.2754*	.02319	.000	.2296	.3211
	Ripe	Under-ripe	−.0629*	.02319	.007	−.1087	−.0172
		Over-ripe	.2124*	.02319	.000	.1667	.2582
	Over-ripe	Under-ripe	−.2754*	.02319	.000	−.3211	−.2296
		Ripe	−.2124*	.02319	.000	−.2582	−.1667
Games-Howell	Under-ripe	Ripe	.0629*	.01860	.003	.0187	.1071
		Over-ripe	.2754*	.02609	.000	.2133	.3374
	Ripe	Under-ripe	−.0629*	.01860	.003	−.1071	−.0187
		Over-ripe	.2124*	.02422	.000	.1547	.2701
	Over-ripe	Under-ripe	−.2754*	.02609	.000	−.3374	−.2133
		Ripe	−.2124*	.02422	.000	−.2701	−.1547

Based on observed means. The error term is mean square (error) = .016.
* The mean difference is significant at the .05 level.

Table 17
95% confidence intervals of pair-wise differences in mean changes in oil palm FFB categories.

Oil palm FFB categories	*M*	SD	Under-ripe	Ripe
Under-ripe	.8290	.11489		
Ripe	.7661	.08692	0.0172–0.1087	
Over-ripe	.5537	.16623	0.2296–0.3211	0.1667–0.2582

independent variable, the oil palm FFB categories, included three stages of ripeness, namely under-ripe, ripe, and over-ripe. For the anthocyanin as a dependent variable, the assumption of homogeneity of variance (Levene's test) has been met, $F(2177) = 116.65$, p equal to 0.249 (Table 4). The p value is located in the column labelled Sig. The p value is more than 0.05, so we accept the null hypothesis that the error variance of the dependent variable is equal across groups. Since the first rule has been obeyed, the Welch and Brown–Forsythe *F*s results, as shown in Table 5, can be ignored. The strength of relationship between the oil palm FFB categories and anthocyanin content, as assessed by r^2, was strong; with the oil palm FFB categories factor accounting for 71.8% of the variance of the dependent variable (Table 6). The main ANOVA summary, as shown in Table 7, exemplifies that the observed significance value is less than 0.05, so there is a significant effect among the oil palm FFB ripeness categories on anthocyanin content.

Follow-up tests were conducted to evaluate pair-wise differences among the means. Because the variances among the three categories ranged from 0.007 to 0.012 (Table 8), we chose to assume that the variances were homogenous and conducted post-hoc comparisons using the LSD test (a test that assumes equal variances) among the three groups. The test of homogeneity of variances was non-significant, $p = 0.249$. This confirmed that the population variances were equal and this allows the use of a multiple comparison procedure. The results of the LSD test are shown in Table 9. There was a significant difference in the means between all categories. In the table labelled Multiple Comparisons, the star (*) in the 'mean difference' column indicates which comparisons were significant. The 95% confidence intervals for the pair-wise differences, as well as the means and standard deviations for the three oil palm FFB categories, are shown in Table 10. A Gomes-Howell test was performed to give a strong argument that there was significant difference among oil palm FFB ripeness categories (Table 9).

2.10.2. Statistical analysis for flavonoids

The same statistical analysis procedure was performed for the flavonoid content. The error bar chart of the flavonol data (Fig. 6) illustrates the magnitude of the mean for each category of oil palm FFB; the 'I' shapes show the confidence interval of these means. The error bar shows that there was very little variance across samples. The independent variable, the oil palm FFB categories, included three stages of ripeness, namely under-ripe, ripe, and over-ripe. For the flavonoids as a dependent variable, the assumption of homogeneity of variance was violated because the homogeneity test was significant, $F(2,177) = 96$, $p < 0.01$ (Table 11). The p value is located in the column labelled Sig. The p value is less than 0.05, so we reject the null hypothesis that the error variance of the dependent variable is equal across groups. The main ANOVA table (Table 12) explains that because the observed significance value is less than 0.05, there was a significant effect of flavonol content of oil palm FFB ripeness categories. Further testing using Welch and Brown–Forsythe *F*s was performed (Table 13). Test conclusions remain the same; both *F*s have significance values less than 0.05. The strength of relationship between the oil palm FFB categories and anthocyanin content,

Table 18
Accuracy assessment for the overall classification model.

Model	Predictors		
	Anthocyanin	Flavonoids	Anthocyanin and flavonoids
Stochastic gradient boosting trees	84.4%	59.8%	87.7%
Interactive tree (C&RT)	79.4%	64.4%	79.4%
General stepwise linear discriminant analysis	74.4%	57.2%	74.4%
MARSplines	74.4%	–	74.4%
STATISTICA automated neural network	77.2%	61.9%	78.3%
Support vector machine	75.0%	60.6%	76.7%
Naïve Bayes classifiers	73.6%	60.6%	75.6%
K-nearest neighbours	75.0%	63.3%	80.0%

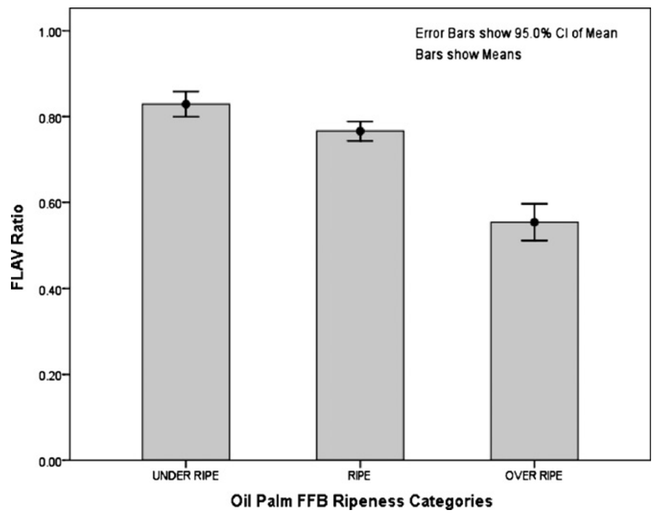

Fig. 6. The error bars chart for FLAV ratio with different oil palm FFB ripeness categories.

as assessed by r^2, was strong, with the oil palm FFB categories factor accounting for 46.1% of the variance of dependent variable (Table 14).

A follow-up test was conducted to evaluate pair-wise differences among the means. Because the variances among the three categories ranged from 0.008 to 0.028 (Table 15), we chose to assume that the variances were homogenous and conducted post-hoc comparisons using the LSD test (a test that assumes equal variances) among the three groups. The test for homogeneity of variances was not significant, $p < .01$ but was already backed up by a significant Welch and Brown–Forsythe *F*s test. This confirms the population variances to be equal and allows for a LSD test. Results from the post-hoc comparisons are shown in Table 16. There was a significant difference in the means between all categories. In the table labelled Multiple Comparisons, the star (*) in the Mean Difference column indicates the comparisons that were significant. The 95% confidence intervals for the pair-wise differences, as well as the means and standard deviations for the three oil palm FFB categories, are shown in Table 17. A Gomes-Howell test was performed to give a strong argument that there really was a significant difference among oil palm FFB ripeness categories (Table 16).

2.11. Classification analysis

The summary of overall classification is shown in Table 18. The best classification accuracy of 87.7% uses a Stochastic Gradient Boosting Trees model, and anthocyanins and flavonoids, as predictors. The other models, using single and interaction predictors, gave varying classification accuracy ranging from 57.2 to 84.4%. Based on these classification results, anthocyanin is a better predictor than flavonoids. The highest accuracy using anthocyanin as a predictor was 84.4%, when using a Stochastic Gradient Boosting Trees method. As discussed previously, red colouring (due to the anthocyanin pigment) has a high potential to differentiate every ripeness category and each oil palm FFB category clearly had different amounts of anthocyanin content.

3. Conclusions

The results show that flavonoid and anthocyanin content decreases from immature to over-mature oil palm FFB. Both of these phenolic contents had similar behaviours where actively operating during the ripe stage, and were slightly decreasing when approaching the ripe and over-ripe stage. The overall relationship between contents based on Pearson' correlation was r^2 is 0.84. Statistical analysis using analysis of variance (ANOVA) and pair-wise testing proved that both predictors gave a significant different among under-ripe, ripe, and over-ripe categories of oil palm FFB. This shows that both predictors can be used as good indicators to classify oil palm FFB. The classification analysis was performed by using both predictors together and separately, by several methods. The highest overall classification accuracy was 87.7% using a Stochastic Gradient Boosting Trees model for both predictors. The other classification methods, using either independent or both predictors together, gave various results ranging from 57.7 to 84.4%. This research has identified the potential of a fluorescence sensor to be used in the grading task, and will help in the future development of an automatic oil palm FFB grading system.

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