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Use of Portable Fluorescence Spectroscopy and SIMCA Method to Test The Authenticity of *Apis mellifera* Honey From Coffee Flower Nectar Mixed With Two Artificial Sweeteners

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ABSTRACT

Honey with coffee flowers nectar is native honey formed from flower nectar. In this investigation, corn syrup and rice syrup, two artificial sweeteners, were utilized as an adulterant. Portable fluorescence spectroscopy and the SIMCA method are the tools and techniques employed. There were up to 20 samples of pure Apis mellifera honey and up to 120 samples of mixed honey (MC), each used twice. Data on the emission spectra, which are excited at a wavelength of 365 nm, were measured over the wavelength range of 300-800 nm. To improve accuracy, sensitivity, and specificity, the original spectral data was altered using a number of pre-treatments. Pretreatment with the original data with smoothing moving average may accurately identify samples and provides 100% accuracy, sensitivity, and specificity. One of the steps of the SIMCA approach, the cumulative PC, has a value of 92%, which indicates that it well explains the variation of the data. The x-loading plot's values are near the peak of the waves at 378 and 460 nm, indicating the existence of phenolic and flavonoid chemicals at those wavelengths.

1. INTRODUCTION

Honey is a high value product and contains carbohydrates (70-80% w/w), water (10-20% w/w), organic acids, enzymes, vitamins, and protein (Baroni *et al*., 2015). The main components in honey carbohydrates are glucose and fructose. The high levels of glucose and fructose contained in honey form hydrogen bonds in water and play a role in maintaining skin hydration. There are several aspects that can determine the quality of honey, for example honey color, pH, viscosity, pH, electrical conductivity and water content (Apriani *et al*., 2013). Honey is also a food stuff that has a sweet and thick taste, golden to dark brown in color with high sugar content and low fat (Wulansari, 2018). The composition of honey is greatly influenced by the type of bee and the origin of the honey nectar that produces the honey (da Silva et al., 2016). In Indonesia, honey has its own quality standards as set by the National Standardization Agency (BSN), starting from the

beginning of harvesting to the packaging process. Honey has also determined water content, acidity, taste and metal contamination (BSN, 2013).

Honey with coffee blossom nectar is honey which is formed from flower nectar which is dominated by coffee flowers which are sucked by livestock honey bees which are deliberately cultivated on coffee plantations. The Apis mellifera bee species is often cultivated during the flowering season on coffee plants in coffee plantations. In Indonesia, there are often cases of adulterated honey content which causes the natural sweet taste of the honey consumed to be lost. Because the process is so long, a lot of money has to be spent to get honey with naturally sweet content. It can be seen from the composition of real honey, there are many producers who deliberately mix glucose and fructose syrup with honey because the main ingredient in honey is carbohydrates, which are actually not suitable or suitable for harvesting. This is done to get maximum profits with the capital spent. One example of another case occurred in Indonesia, where it was circulated that 3 people had practiced adulterating honey by using a mixture of sweeteners, one of which was corn syrup (Felim, 2021). Syrup is a drink made from a mixture of sugar and water with a sugar solution of at least 65% and additional components that are permitted according to the guidelines set by the SNI 8664 standard (BSN, 2018). HFCS or High Fructose Corn Syrup is made by hydrolyzing corn starch, which contains amylose and amylopectin, chemically and enzymatically. High fructose corn is made from corn syrup, which consists mostly of fructose. With the help of the enzyme fructose isomerase, high fructose corn syrup is converted into high fructose corn syrup (HFCS). This enzyme functions as a catalyst in the conversion of high glucose corn syrup to HFCS. The resulting HFCS has a fructose content of 42%, while HFCS with a fructose content of up to 90% is produced using a fractionation process which largely removes the remaining glucose from the 42% HFCS. HFCS 90% and HFCS 42% are combined to make HFCS 55% (Aulia, 2022).

Rice syrup, also known as RMS (Rice Malt Syrup) is produced using only brown rice. The rice is first cultured with enzymes to break down the starch, and then the mixture is cooked until it reaches a syrupy consistency. The finished product has small amounts of glucose, as well as soluble complex carbohydrates, maltose and other sugars.

The use of ultraviolet (UV) spectroscopy is an example of a spectroscopic technique that has the potential to be developed in Indonesia. The spectroscopic method is relatively accurate during research, can be operated easily, requires minimal preparation for sample testing, and most importantly, this tool has a very affordable price for conducting research, making it possible for downstream technology processes to be implemented. In the modern era like now, broadly speaking, honey authenticity testing methods are divided into 5. Examples are chromatography, mass spectrometry (MS), infrared spectroscopy, NMR (nuclear magnetic resonance) and molecular technology (Chin & Sowndhararajan, 2020). A spectroscopic approach based on UV spectroscopy has been tried in Indonesia to verify the authenticity of agricultural products such as coffee and tea. For example, research conducted by Aulia (2022) used spectroscopy to identify adulteration of stingless bee monoflora honey mixed with the sweetener HFCS-55 and Himawan (2022) used spectroscopy to study the mixing of stingless honey mixed with corn syrup. Then Hadi *et al*. (2017) used spectroscopy to determine polyphenol levels in packaged tea extracts.

Outside Indonesia, there are examples, namely Wang *et al*. (2019) used a combination of GC (gas chromatography) and MS (mass spectometry) methods to differentiate Apis mellifera and Apis cerana honey. Then there are examples of molecular techniques, such as PCR (polymerase chain reaction), to test the authenticity

of orange honey (Citrus spp.) mixed with sweetener from rice molasses (Sobrino-Gregorio *et al*., 2019). These methods have been tested and are widely available in Indonesia. The disadvantage of benchtop spectroscopy is that it is less efficient, we have to take a sample first to test the sample. This tool also has a heavy weight and large size, making it an inefficient benchtop spectroscopy tool. Due to the development of the times, especially in the field of technology, a tool has been created to make it easier for researchers to carry out their research. This tool is a portable spectroscope which has a small size, is easy to carry anywhere and can be held or can be called a handheld so it is more efficient and more sensitive. in collecting data to test the levels of a substance, for example honey. This research used a portable spectroscopic instrument and two sweeteners because in previous studies on average only one sweetener was used. So this research was carried out to identify the authenticity of coffee honey and honey mixed with sweeteners to see the differences produced by the SIMCA method.

2. MATERIALS AND METHODS

2.1. Time and Place

This research was carried out in January 2023, at the Bioprocess and Post-Harvest Engineering Laboratory, Department of Agricultural Engineering, Faculty of Agriculture, University of Lampung. The method used is the SIMCA method.

2.2. Tools and Materials

The tool used in this research is a portable spectroscope with emission spectra measured in the wavelength range 300-800 nm which is excited at a wavelength of 365 nm. The sample used was Apis mellifera honey with coffee flower nectar from the Sarang Maduku shop and which was harvested on the slopes of Mount Muria, Central Java. The sweeteners used in this research were corn syrup and rice syrup. These two sweeteners are a mixture of pure Apis mellifera honey which will be tested for authenticity and distilled water as a diluent.

2.3. Research Methods

The research method used in this research is the Soft Independent Modeling of Class Analogies (SIMCA) method. SIMCA is a multivariate analysis technique used to test the power of discrimination and classification on a sample being tested. Before testing the discriminatory power of samples, first build a PCA model to identify the causes of differences between samples, determine the variables that play a major role in sample differences and be able to quantify some information, both useful and inappropriate information (noise) that will be released in the form of data (Suhandy & Yulia , 2019). SIMCA model development was used to evaluate the PCA model. The evaluation model is a confusion matrix with formula as presented in Table 1.

Table 1. Confusion matrix

Class type	Class A (actual)	Class B (actual)
SIMCA A	a (TP)	b (FP)
SIMCA B	c (FN)	d (TN)

Based on the confusion matrix table, the calculation of accuracy, sensitivity, specificity and error values is then carried out using the following equation (Lavine

2009; Suhandy & Yulia 2019). The calculation was performed as the following:

$$\text{Accuracy} \quad (AC) = \frac{a+d}{a+b+c+d} \times 100 \tag{1}$$

$$\text{Sensitivity} \quad (S) = \frac{d}{b+d} \times 100 \tag{2}$$

$$\text{Specificity} \quad (SP) = \frac{a}{a+c} \times 100 \tag{3}$$

$$\text{Error} \quad (E) = \frac{b+c}{a+d+b+c} \times 100 \tag{4}$$

where *TP* is true positive, *TN* is true negative, *FP* is false positive, *FN* is false negative, *a* is class A samples that fall into class A (TP), *b* is class A samples that are included in class B (FP), *c* is class B samples that are included in class A (FN), *d* is class B samples that are included in class B (TN), class A is pure *Apis mellifera* honey sample, class B is sample of *Apis mellifera* honey mixed with artificial sweeteners.

The calculated values indicate the performance of the classification model. The higher the accuracy, sensitivity and specificity values, the better the classification model that is built. Meanwhile, the false alarm rate shows the level of error in the performance of the classification model, so the lower the false alarm rate value, the better the classification model that is built. MMC has a value of -1 to +1. The MMC value = +1 means perfect classification results (Apratiwi, 2016; de Santana *et al*., 2018). Accuracy values have the following diagnostic levels:

a. Accuracy 0.90 – 1.00 = excellent classification.
b. Accuracy 0.80 – 0.90 = good classification.
c. Accuracy 0.70 – 0.80 = fair or acceptable classification.
d. Accuracy 0.60 – 0.70 = poor classification.
e. Accuracy 0.50 – 0.60 = classification failure.

3. RESULTS AND DISCUSSION

The spectra shown are spectra from measurements from samples of original honey (MA) and mixed honey (MC) which are divided into several classes based on their mixing. In the discussion, the name original data means using data that has been obtained and then processing the data without any special treatment. Meanwhile, pretreatment data means data that has undergone special treatment to avoid noise in the spectra. The pretreatment data used is original data + smoothing moving average 9 segments. Because this data is the best data produced in the pretreatment process.

3.1. Spectral Analysis of *Apis mellifera* Honey

Figure 1 is the spectrum of honey mixed with artificial sweeteners from original data (a) and pretreatment data (b). Both spectra have the same wave peak, namely at 380 nm. At the peak of this wave, it is thought that there are flavonoid compounds and phenolic acids (Markham, 1988). And this wavelength is partly due to the presence of amino acid compounds such as tryptophan, tyrosine, phenylalanine and phenolic acids (Parri *et al*., 2020).

Variations in the mixing level between honey and sweeteners affect the fluorescence emission values. From this graph, it can be seen that the higher the mixing percentage of MA (Real Honey without admixtures), the greater the value of the fluorescence emission. The graph can also be seen in. The tool for measuring the fluorescence value is a portable spectroscope. This tool is a tool that is easy to carry,

small and very efficient for researchers. Portable spectroscopy is a tool that can produce measurement values in the form of fluorescence data.

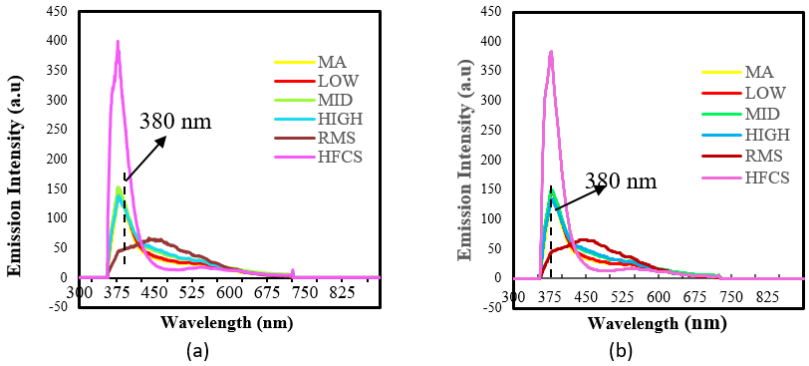

Figure 1. Average spectra: (a) original data and (b) pre-treated data

3.2. PCA Analysis Results

Figure 2 (a) displays the score plot of the results of PCA analysis of original data on pure Apis Mellifera (MA) honey samples and honey samples mixed with 2 sweeteners based on different mixing levels (low, mid and high). The results of the PCA score plot show that the PC-1 and PC-2 values explain the data variance and characterize the sample by 59% and 33%. The cumulative value of PC-1 and PC-2 is 92%. Then in Figure 2 (b) displays the score plot of the results of PCA analysis of original data + smoothing moving average 9 segments on pure Apis Mellifera (MA) honey samples and honey samples mixed with 2 sweeteners based on different mixing levels (low, mid , and high). The result is a PC-1 value of 60% and PC-2 of 33%. If cumulative, it gets a value of 93%. According to Bayu (2015), if the cumulative value of PC-1 and PC-2 exceeds 70%, then PCA analysis is able to explain data variance without having to use PC-3 to increase the cumulative value. In the original data, it has exceeded 70% so it can be said that the PCA score plot is able to explain the data variance.

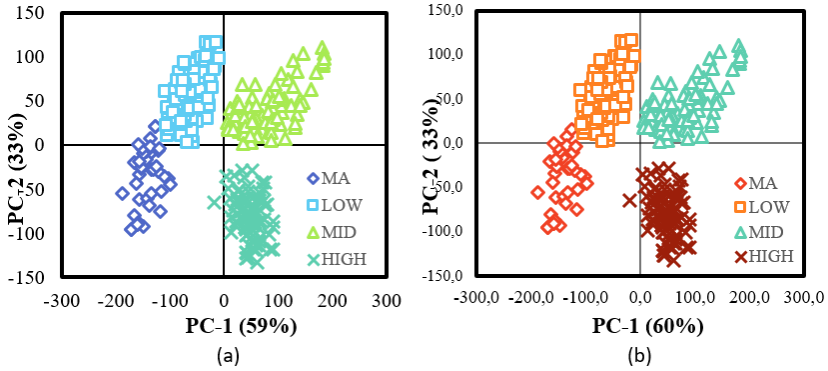

Figure 2. (a) PCA results of original data and (b) PCA results of pretreatment data

Based on Figure 2 shown, it can be seen that pure honey has a value in quadrant III. Meanwhile, mixed honey has different values for each class, the low class is in quadrant II, the mid class is in quadrant I and the high class is in quadrant IV.

In PCA data processing there are results other than plot scores, other results are called X-loadings. Plot scores and X-loadings have complementary values in explaining the correlation of variables. X-loadings can be seen in Figure 3. Figure 3 has a wave

peak at a certain wavelength which can be assumed to indicate a response from a compound. According to Markham (1988) the typical spectrum of flavonoids is at a wavelength between 350-385 nm and is of the flavonol type.

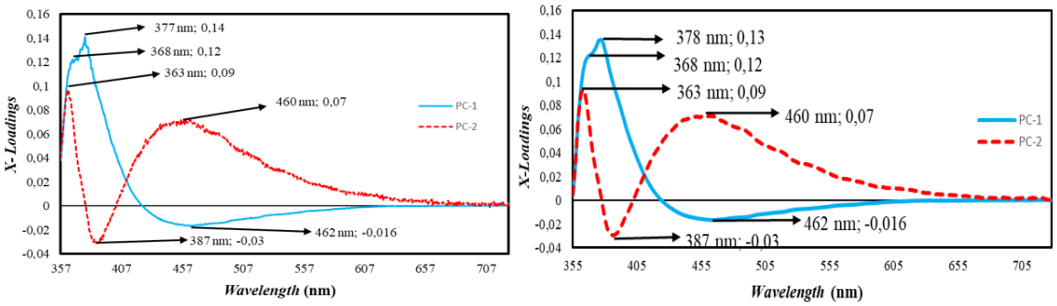

Figure 3. (a) X-Loadings of original data and (b) X-Loadings of pretreatment data

3.3. SIMCA Model Development Results

SIMCA classification is a classification carried out based on the PCA method, so in general SIMCA consists of several stages, the first is Modelling (model development) and the second is Classifying (classification). The following is the modelling stage in the form of PCA from MA and MC samples on original data and data that has been pre-treated to reduce existing noise.

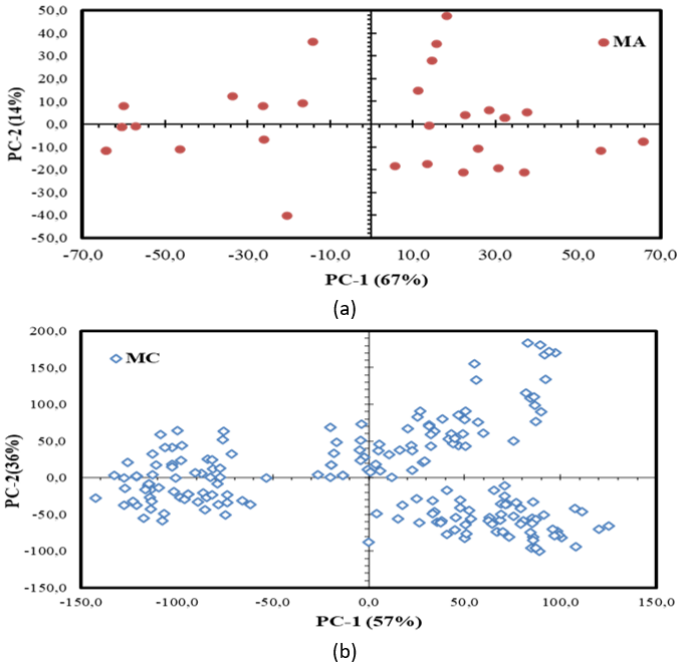

Figure 4. (a) Model SIMCA of original data: SIMCA MA (top), and SIMCA MC (bottom)

In Figures 4 and 5 are images of the SIMCA MA and MC results from each data (original and pre-treated). These data have been said to be good, having an accumulative PC value exceeding the percentage of 70%, as said by Bayu (2015). Because the original data has an accumulative PC MA value of 81% and MC of 93%. Meanwhile, the pre-treated data obtained an accumulative PC MA value of 85% and MC of 95%.

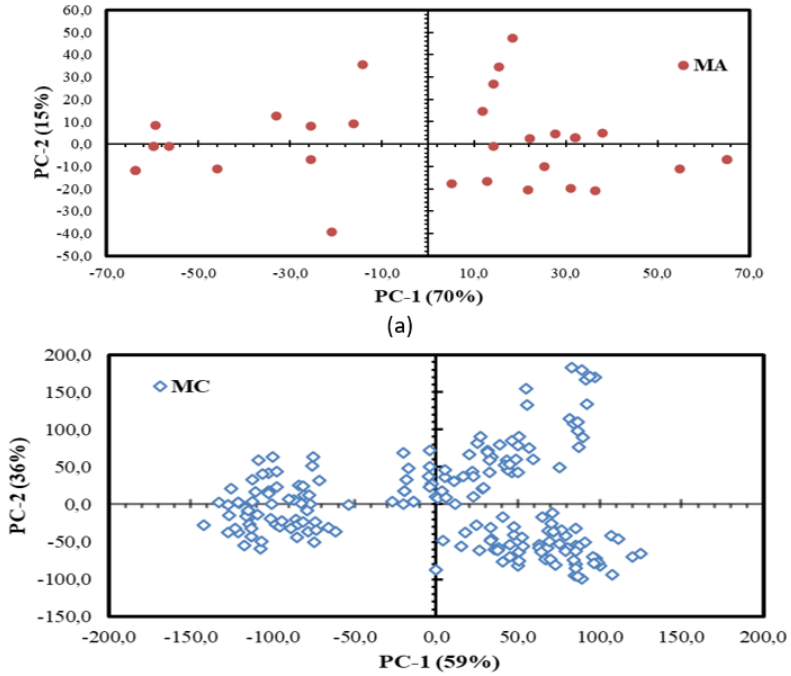

Figure 5. (a) Model SIMCA of the pre-treated data: SIMCA MA (top), and SIMCA MC (bottom)

3.4. Classification Using New Samples (Prediction Samples)

The next stage of developing the SIMCA model is classifying it, so that the boundaries between classes can be seen. In this study, we classified MA samples and MC samples from each sample to prove that the sample was included in its own class. There are four possible sample classification results, namely TP (true positive) where class A samples are classified correctly and are included in class A samples. TN (true negative) where class B samples are correctly classified and included in class B samples. FP (false positive) where the class A sample is classified incorrectly and is included in the class B sample. FN (false negative) where the class B sample is classified incorrectly and is included in the class A sample.

In the original data the classification uses two SIMCA models, namely the MA class model and the MC class model. The prediction samples used consist of 8 MA prediction samples and 48 MC prediction samples. The results of this classification show that there are 7 samples as TP, 1 sample as FP, 3 samples as FN, and 45 samples as TN which can be seen in Table 2.

Table 2. Results of SIMCA MA and SIMCA MC for the original data

Class type	MA class (actual)	MC class (actual)
Result of SIMCA MA	8 (TP)	0 (FP)
Result of SIMCA MC	3 (FN)	45 (TN)

The next stage of grouping according to class is to calculate several values to see the performance of each data, including accuracy, sensitivity, specificity and error as follows:

$$\text{Accuracy (\%)} = \frac{\text{TP+TN}}{TP+FP+FN+TN} \times 100 = \frac{8+45}{8+0+3+45} \times 100 = 94.6\%$$

$$\text{Sensitivity (\%)} = \frac{TN}{FP+TN} \times 100 = \frac{45}{0+45} \times 100 = 100\%$$

$$\text{Specifity (\%)} = \frac{TP}{TP+FN} \times 100 = \frac{8}{8+3} \times 100 = 72.7\%$$

$$\text{Error (\%)} = \frac{FP+FN}{TP+TN+FP+FN} \times 100 = \frac{0+3}{8+45+0+3} \times 100 = 5.4\%$$

Then in the pre-treated data it was found that 8 samples entered as TP, 0 samples as FP, 0 samples as FN, and 48 samples as TN which can be seen in Table 3.

Table 3. Results of SIMCA MA and SIMCA MC for the pre-treated data

Class type	MA class (actual)	MC class (actual)
Result of SIMCA MA	8 (TP)	0 (FP)
Result of SIMCA MC	0 (FN)	48 (TN)

The next step of grouping according to class is to calculate several values to see the performance of each data, including accuracy, sensitivity, specificity, and error as in the following:

$$\text{Accuracy (\%)} = \frac{\text{TP+TN}}{TP+FP+FN+TN} \times 100 = \frac{8+48}{8+0+0+48} \times 100 = 100\%$$

$$\text{Sensitivity (\%)} = \frac{TN}{FP+TN} \times 100 = \frac{48}{0+48} \times 100 = 100\%$$

$$\text{Specifity (\%)} = \frac{TP}{TP+FN} \times 100 = \frac{8}{8+0} \times 100 = 100\%$$

$$\text{Error (\%)} = \frac{FP+FN}{TP+TN+FP+FN} \times 100 = \frac{0+0}{8+48+0+0} \times 100 = 0\%$$

Data from the confusion matrix calculation using pre-treated data produces an accuracy value of 100%, sensitivity of 100%, specificity of 100% and error of 0%. Based on the values produced in the pre-treated data, it shows that the model built has classified correctly and can be said to be a very good classification.

3.5. Coomans Plot

The Coomans plot is one of the results or output of SIMCA to explain the distance between the sample and its class model (Si). The closer the distance between the sample and the class model, the more accurately the sample is categorized as a member of that class. The result of the Coomans plot is a plot that has 2 membership lines to divide each class. We can see the Coomans plot of the original data and pretreatment data in Figure 6.

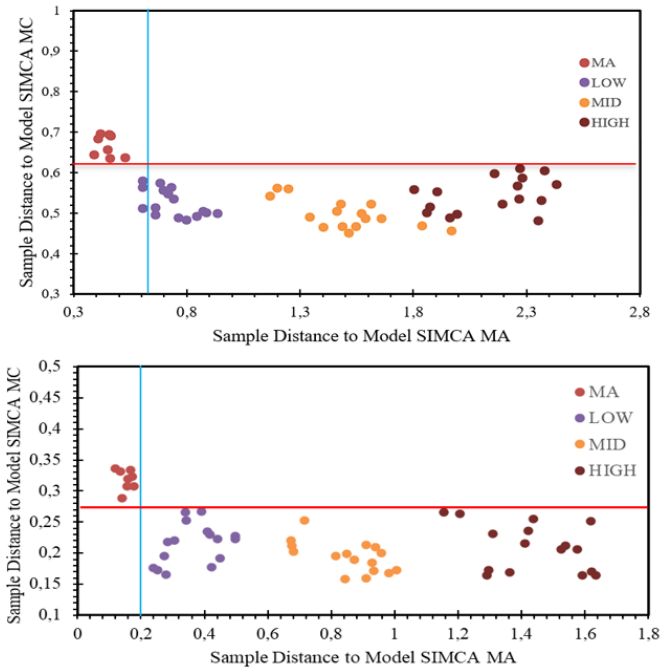

Figure 6. Coomans plot of original data (top), and pre-treated data (bottom)

3.6. Receiver Operating Characteristic (ROC) Curve

The Receiver Operating Characteristic (ROC) curve is the relationship between specificity and sensitivity analysis. The ROC curve is useful for presenting visually, organizing and being able to determine the best classification method based on performance. The ROC curve is the result of a plot between true signal (sensitivity) and false signal (1-Specificity). The ROC curve can be seen in Figure 7.

In the original ROC curve, it can be seen that each significance level has a different 1 -SP relationship and sensitivity. Only at the significance level of 0.1, 0.5, 1, 5, 10% does the relationship between 1-SP and sensitivity have the same. Then, in the pre-treated data, the classification results at each significance level have the same classification performance or have a relationship between sensitivity and specificity with the same value, namely 1. Based on these results, it can be said that at the MA and MC classification significance levels the percentage is 0.1, 0 .5, 1, 5, 10, and 25% which have a value at coordinates (0, 1) so they are classified as very good or excellent classification.

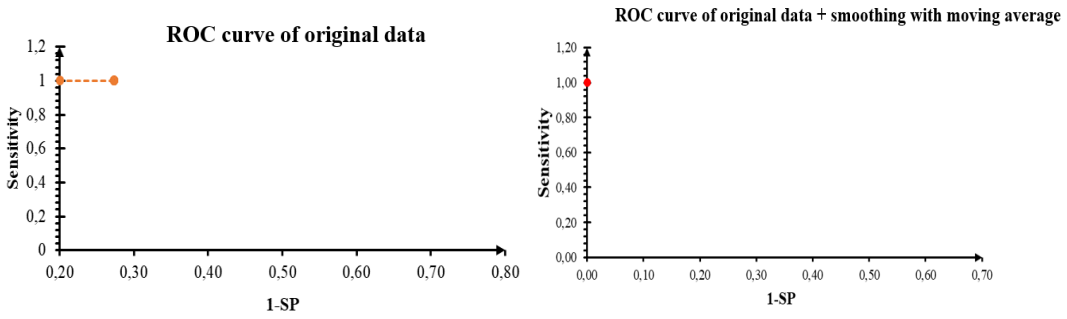

Figure 7. ROC curve based on MA and MC classes: original data (left), and pre-treated data (right)

4. CONCLUSION

The results of this research can prove that using the portable spectroscopy analysis method and the SIMCA method can test for adulteration of *Apis mellifera* honey with coffee flower nectar mixed with two sweeteners, namely Rice Malt Syrup (RMS) and High Fructose Corn Syrup (HFCS). The SIMCA method used can produce good data in processing. The performance of the SIMCA model obtained is the value of accuracy, sensitivity, specificity and error on the original data with a value of 94.6%; 100%; 72.7%; and 5.4%, respectively. Meanwhile, the pre-treated data is classified as very good, namely 100% in all values. The PCA model that was built could explain the data variance cumulatively by 92% for original data and 93% for pre-treated data.

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