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Detection and Classification of Honey Adulteration Combined with Multivariate Analysis

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Abstract: Honey is a natural sweetener with a yellowish substance made up of bee secretions and plant nectar extracts. Main composition of honey are sugars or carbohydrates and water in the chemical composition and contain a great number of minor components such as minerals, amino acids, proteins, acidity, and pH. Honey adulteration is a global concern due to lack of awareness of people and policies. There is a various method that has been conducted to detect honey adulteration such as SCIRA, DSC, FTIR, NIRS, and NMR and these methods mostly used multivariate data analysis to classify the adulteration of honey. PCA is the most used technique in the classification of honey adulteration where the data obtained is clustered according to adulteration level and type of the adulterant. This paper explains on different methods to detect honey adulteration and common technique used on classification of honey. It can be concluded that PCA is the most used technique based on different method of honey adulteration detection.

Keywords: Honey adulteration, detection of honey adulteration, DSC, PCA

1. Introduction

Honey is a yellowish substance made up of bee secretions and plant nectar extracts. Some bee species visit plant nectar, collect and store the extract as food [1]. Various types of plant sources, such as Gelam, pineapple, mango, coconut or Kelulut honey, can result in different kinds of honey [2]. As an entirely natural food, honey is popular all over the world because of its nutritional value and bioactive properties. Honey is usually used as a sweetener in the food industry and is also introduced into cosmetics [3]. Other than a sweetener, honey also has a medicinal benefit that can be used as a wound healing dressing, contains antimicrobial agents and strong antioxidant properties, and is good enough to treat obesity, diabetes, and cancers. The diverse uses of honey known to consumer contribute to higher retail demand causes higher needed in production and market of honey [2]. Hence, become the main target of food adulteration due its popularity and high demand [4]. Adulterant such as sugar syrup that usually added are high fructose corn syrups (HFCS), high fructose inulin syrups (HFIS), invert syrups (IS) and corn syrups (CS) [1]. Adulteration of honey is addition of cheap foreign materials called adulterant into pure honey causes decreases in its nutrition and medicinal value [5].

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The main composition of honey are sugars or carbohydrates and water in the chemical composition and contain minor components such as minerals, amino acids, proteins, acidity, and pH. Above all, sugars are the main constituents of honey, comprising about 95 % of honey dry weight with fructose and glucose included and 23% water moisture [1]. Honey's stability against fermentation and granulation is determined by the amount of water it contains [6]. The minor component, acidity content, is rather low, yet it is critical for the honey flavor. The predominant acid is gluconic acid, a result of glucose oxidation by glucose oxidase, which is largely added by the bee itself. [7].

The international Codex Alimentarius has specified the identification and quality requirements for honey authenticity. It takes into account the sensory and physiochemical qualities of honey based on these regulations [8]. Moisture content, sucrose content, and reducing sugars content, pH value, electrical conductivity, ash content, free acidity, diastase activity, and hydroxymethyl furfural (HMF) content are all major quality parameters based on regulations [9]. The quality of honey can be degrading and ferment easily when moisture content is high, low density, and high electrical [1]. Water moisture content is an important characteristic because it can affect the physical properties of honey such as viscosity and crystallization, colour, flavour, taste, specific gravity, solubility, and conservation. Based on the Codex Standard for Honey, the moisture content in honey should not exceed 20 g 100 g^{-1}[8].

There are many different methods proposed to detect honey adulteration include Stable Carbon Isotope Ratio Analysis (SCIRA) [10], Differential Scanning Calorimetry (DSC) [11], Surface Plasmon Resonance (SPR) [12], Nuclear Magnetic Resonance (NMR) [3], and Liquid Chromatography (LC) [9]. Other method includes High Performances Liquid Chromatography (HPLC)\ [4],[13], Fiber optic displacement sensor (FODS) [14], Electrical Impedance Spectroscopy[15], Electronic Tongue (ET) [16], Electronic Nose (EN)[10], and Near-Infrared Spectroscopy (NIRS)[11]. The aim of this research is to study on the different method to detect honey adulteration and the most common data analysis technique used for classification of honey adulteration.

2. Materials and Methods

2.1 Method to Detect Adulteration of Honey

2.1.1 Stable Carbon Isotope Ratio Analysis (SCIRA)

All SCIRA is one of the earliest uses procedures for detecting honey adulterants, and it is still practiced today. This method relying on $^{13}C/^{12}C$ isotope ratio[17]. Based on M. Tosun [18], it is stated that SCIRA method is useful in detecting sugar cane or corn syrup adulterant in honey[18]. This method principle is based on the differences between the $^{13}C/^{12}C$ ratio of C_4 originating from monocotyledonous species of sugar cane and corn when compared to dicotyledon species which is C_3 plant [19]. This method can be expressed as equation follows:

$$^{13}C/^{12}C = \delta^{13}\ (\%) \quad (1)$$

According to A. Guler et al., to be considered pure honey, the difference between the δ^{13} C values of protein and honey should not exceed 1%, and the δ^{13} C value of honey should be more negative than -23.5% [19]. However, there are companies that adjusted and improved their technique in adding adulterant into honey by blending artificial sweeteners with honey that had $\delta^{13}C$ less than -23.5%. So, a study found a method called Internal Standard Carbon Isotope Ratio Analysis (ISCIRA) where it detects honey adulteration by comparing the differences in $^{13}C/^{12}C$ ratio between the sugar and protein. The percentage of the adulteration can also be estimated using the following formula [20]:

$$Adulteration, \% = \frac{(\delta\%\ \text{Prot.} - \delta\%\ \text{Honey})}{(\delta\%\ \text{Prot.} - \delta\%\ \text{Sweetener})} \text{x} 100 \quad (2)$$

2.1.2 Electronic Nose (EN)

EN is a bio-mimicking sensors that based on mammalian olfactory system. This method is capable of recognizing the mixture of organic sample [21]. EN can determine the fingerprint data and by compiling this data, a discriminant model will be established. Even an unknown samples origin can be determined by this model as study perform[10]. According to A. Zakaria, A. Shakaff and M. Masnan [22], 32 non-selective sensors made of various polymer matrixes were combined with a carbon block. As mentioned before, these sensors array combine with suitable pattern recognition algorithm can mimic the human olfaction system such as principal component analysis (PCA) and linear discriminant analysis (LDA) that is used in this paper [22]. Sample of honey is kept in a test tube that sealed with silicone stopper. To measure the data, these sample is then heated up for 10 minutes on the heater block to generate sufficient headspace volatile[22]. Then, the sensor array will respond to the aroma that contained wide range of chemical. The chemical is then convert to electrical signal that will be read by the computer [21]. The data is combined with pattern recognition technique to separate the data. For example LDA technique will separate and clustered the sample based on the origin, sugar syrup and adulterated honey [22].

2.1.3 Electronic Tongue (ET)

ET is a method of pattern recognition and multivariate calibration for data processing which is an analytical instrument that composes of an array of non-specific, poorly selective, and chemical sensors [23]. ET used the same principle as EN [24]. The signal gives out by the sample will be collected by the sensor and processed using the pattern recognition tools to generate models that classify and qualify the sample. There are several alternatives to electronic tongue systems, the voltammetry being one of the most used and Electronic tongue systems are capable of identifying and classifying liquid samples using appropriate multivariate analysis techniques such as PCA, LDA or correlation analysis (CA) [16]. From the study, the data is discriminated using PCA. Honey samples are in the centre of the score graph, while syrup is on the side of the graph, according to the PCA. The findings revealed that this approach can distinguish between all sorts of pure samples tested [16].

2.1.4 Differential Scanning Calorimetry (DSC)

DSC makes it easier to analyse different food components like protein, fat, and carbohydrate [25]. To distinguish between honey and syrups, DSC employed the glass transition temperature. This method was used to study the temperature behaviour of honey [26]. In a study by L. Sobrino-Gregorio, in comparison to a pure honey sample, the addition of sugar syrup causes considerable changes in the thermal properties of the adulterated sample in terms of proportion to the level of adulteration. The thermal properties acquired using a Differential Scanning Calorimeter equipped with an intracooler based on the research. The sample was subjected to a temperature change [25]. ANOVA is applied to study the thermal properties obtained from the sample. The data also analysed using PCA and after applying the PCA, the plot score shows that the adulterated and pure honey can be distinguished based on adulteration level and type of adulterant. Based on the result of the study, the sample is clustered accordingly where the highest adulteration level located far from pure honey and the cane sugar syrup with 5% adulteration level is located near pure honey [25].

2.1.5 Fourier Transform Infrared – Attenuated Total Reflectance (FTIR – ATR) Spectroscopy

FTIR-ATR is a technique used for molecular fingerprinting and a recent study in 2018 proposed that this technique can be enhanced by combining it with chemometric. In this study, for the rapid detection and quantification of honey adulterants, FTIR-ATR spectroscopy in combination with chemometrics is used. In comparison to standard techniques, which take hours to complete, this approach takes only 7–8 minutes to accomplish. The spectral data from both pure and adulterated honey is collected that range between 750-1180 cm^{-1} and for data analysis, a chemometric model was built. The classification of pure and adulterated honey is performed by PCA. The classification can be seen from the PCA score plot. Based on the result of this study, FTIR-ATR with chemometric can classify adulterated concentration above 8% and as low as 2% [27]. This technique combines with chemometric enables the classification of pure and adulterated honey. It is a rapid and non-destructive method as PCA enable to differentiate between pure and adulterated honey [27][28].

2.1.6 Near – Infrared Spectroscopy (NIRS)

NIRS has been previously used for qualification and quantification of honey and the honey sample was examined using this technique in combination with chemometrics [28][29][30]. Previous work has successfully used NIRS in combination with PCA, LDA, and partial least square regression (PLS) to detect different levels of adulteration in honey [32], and there is also a study that uses NIRS in combination with other chemometrics methods such as hierarchical cluster analysis (HCA), LDA, and PLS [31]. In the experiment performed by Ferreiro-González, the NIR spectra were recorded, and all the samples were analysed in the range of 400-2500nm. The data analysed were duplicated and the average spectrum of the sample will be used in multivariate analysis which are HCA, PCA, and LDA to group the sample according to the level of adulteration [29]. There are also worked by Alexandra Rust that uses ANOVA-simultaneous analysis (ASCA) to look at the effects of storage temperature, the presence of sugar syrup adulterant, irradiation treatment, and ageing on NIR spectra. This research aims to quantify and characterize the spectral properties that contribute to those impacts [11].

2.1.7 Low Field Nuclear Magnetic Resonance (LF 1H NMR)

The relaxation time constant T1 and multi exponential relaxation decay in food are studied using the LF 1H NMR method. This signifies that the food contains water. Honey's LF 1H NMR relaxation profile may be modelled as a linear combination of characteristic relaxation times from the detectable hydrogens contained in their structure, according to a study published in 2014. As an adulterant, this study uses pure blossom honey and high fructose syrup. The sample's T2 has been measured, and the LF NMR relaxation curve has been fitted to a multi-exponential curve. The result shows that the adulterate concentration in the honey sample affect the relaxation time significantly as there is a correlation between the relaxation time and pH, water activity, and moisture content are physical-chemical factors [32].

2.2 Classification of honey adulteration

As mentioned before, there are many methods to detect honey adulteration and most of them uses multivariate analysis to analysed the data except for SCIRA method where the honey sample can be classified between pure and adulterated by comparing the percentage of δ^{13} C value of honey should be more negative than -23.5%[19]. In this paper Principal Component Analysis (PCA) will be highlighted as most of the research performed before uses PCA as the data analysis technique.

Data analysis by PCA performed in this study use Trialware Minitab by LLC (version 20.2). PCA was performed to visualize the honey sample clustering based on the level of adulteration and the type of adulterant. The data for this simulation is taken from DSC method paper by Sobrino-gregorio 2017, that included the glass transition temperature from DSC thermograms perform in the research. The study uses sunflower honey sample as pure honey because its glass transition temperature and sugar composition shows intermediate behaviour [25]. The glass transition temperature was measured with various types of syrup and levels of adulteration [25] as shown in Table 1. This set of data was chosen because it covers a wide range of syrup (agave, barley, corn, maple, rice, and sugar cane syrup) with the composition details. Hence, the simulation that aims to classify the adulterated honey based on the type and level of adulteration can be done. Fig. 1 shows the flowchart of this simulation.

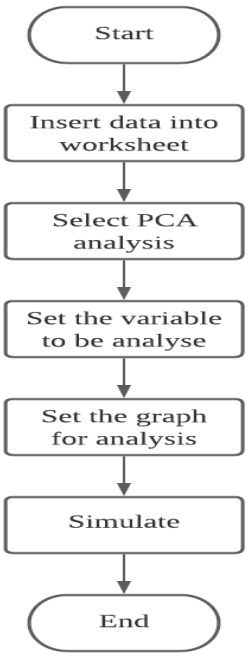

Fig. 1 - Flowchart of the simulation using Minitab software

Table 1 - Data of glass transition temperature and composition of sugar and protein for the syrup and sunflower honey

Syrup	Sample	Glass transition temperature (°C)		Estimated Composition (g/100 g dm)			
		Tgonset2	Tgmidpoint	Fructose	Glucose	Sucrose	Maltose
Pure Honey	Sunflower	65	72	46	40.5	0	0
Agave (A)	H80:A20	68.9	77.8	55.67	34.32	0.06	0
	H90:A10	68	75.7	50.79	37.45	0.03	0
	H95:A5	62.8	70.56	48.38	39	0.02	0
Maple (M)	H80:M20	11.8	17.6	38.58	34	13.85	0
	H90:M10	69.7	77.9	42.37	37.34	6.76	0
	H95:M5	63.9	73.9	44.2	38.96	3.34	0
Rice IV (RIV)	H80:RIV20	66.5	73.84	36.92	42.61	0	7.27
	H90:RIV10	68.7	76.2	41.46	41.57	0	3.63
	H95:RIV5	70.9	80.05	43.73	41.05	0	1.81
Rice I (RI)	H80:RI20	53.9	63.6	36.75	37.27	0	6.43
	H90:RI10	54.5	63.6	41.36	38.9	0	3.22
	H95:RI5	59	69	43.68	39.72	0	1.61
Rice II (RII)	H80:RII20	58.7	69.9	36.83	39.27	0	5.65
	H90:RII10	60.6	71	41.41	39.9	0	2.82
	H95:RII5	59.8	69.7	43.7	40.22	0	1.41
Brown Rice (B	H80:BR20	66	73.8	36.71	33.16	0	10.53
	H90:BR10	57.7	68	41.34	36.84	0	5.27
	H95:BR5	61.9	71.6	43.66	38.69	0	2.64
Rice III (RIII)	H80:RIII20	57	65	36.82	36.52	0	5.43
	H90:RIII10	57.7	67.8	41.4	38.53	0	2.72
	H95:RIII5	59.2	70.5	43.7	39.53	0	1.36
Sugar Cane (SC	H80:SC20	63	70.9	41.92	38.14	6.26	0
	H90:SC10	66.75	76.6	43.96	39.34	3.12	0
	H95:SC5	68.4	77.5	44.97	39.94	1.56	0
Barley (B)	H80:B20	20	26	37.66	37.07	0	12.15
	H90:B10	53.2	62.5	41.83	38.81	0	6.07
	H95:B5	59.9	69	43.91	39.67	0	3.03
Corn (C)	H80:C20	72.9	80.4	38.5	40.69	0	6.66
	H90:C10	67.6	76	42.24	40.61	0	3.34
	H95:C5	68.2	78.5	44.11	40.57	0	1.67

Multivariate analysis was employed for data analysis in the number of studies and this technique allows more than two variables to be studied at the same time [33]. Principle Component Analysis (PCA), the oldest and best-known technique in multivariate analysis. To classify honey adulteration, most of the methods above were integrated with the PCA method. PCA is a technique for identifying patterns in data and classifying them according to their similarities and differences [34].

PCA technique was selected from the multivariate analysis. PCA technique was used because it can create new variables that are linear combinations of the observed variables to identify a smaller number of uncorrelated variables, from a large set of data. This technique reduces the number of variables to make the data easier to analyse. Then, the variable is set based on the data on the worksheet which are Tgonset2, Tgmidpoint, Fructose, Glucose, Sucrose, Maltose, and Protein as shown in Fig. 2.

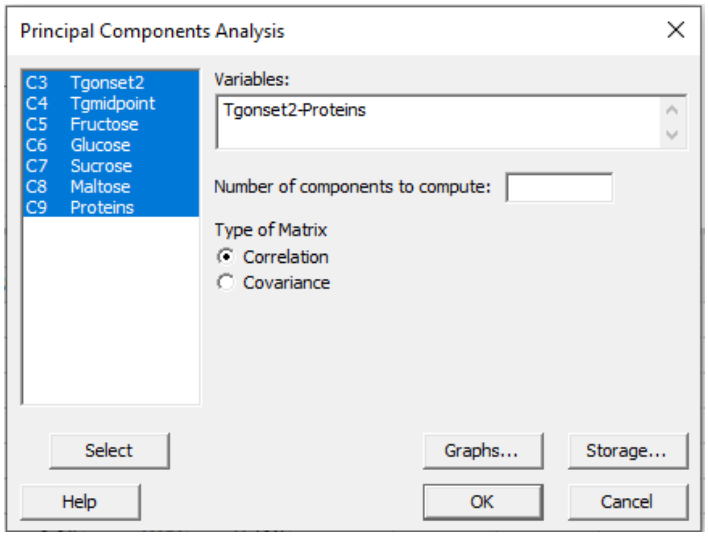

Fig. 2 - The variable that used to perform this Principal Component Analysis

Then, the output graph or plot is picked to analyse the data. In this simulation, the score plot, loading plot, and biplot were chosen to carry this simulation as shown in Fig. 3. This type of graph was chosen since the classification will be done based on the level of adulteration and types of syrup. Score plot output shows the cluster, trends, and outliers in the first two components which are Tgonset2 and Tgmidpoint. For biplot. It will overlay the score plot and the loading plot on the same graph. In this biplot we can analyse the position of each sample. Then, start the simulation where the PCA plot will be obtained.

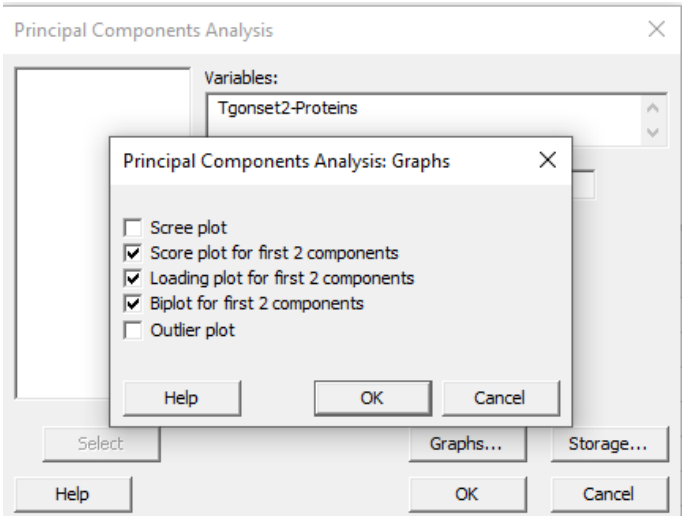

Fig. 3 - Types of graphs used in this simulation using Minitab software

3. Result and Discussion

3.1 Principal Component Analysis (PCA)

The PCA technique was simulated using Minitab software. The Eigenanalysis of the correlation matrix was obtained via the simulation with the results as shown in Table 2. The amount of variation explained by each principal component (PC) can be determined based on the eigenvalue or the cumulative. An eigenvalue that is greater than 1 indicates that PCs account for more variance than accounted for by one of the original variables in the standardized data. Hence, the PCs with eigenvalue greater than 1 will be retained. However, for cumulative proportion, the acceptable variance has to be more than 90%.

As shown in Table 2, the eigenvalues of the first two components were greater than one. In this case, the significant PCA number is taken as two. Moreover, the first four principal components explain 96% of the variation in the data. Therefore, these four principal components are significant and can be used to analyze the honey adulteration.

Table 2 - Eigenanalysis of the Correlation Matrix using Minitab software

Eigenvalue	3.1688	1.9026	0.9208	0.7287	0.1989	0.0779	0.0023
Proportion	0.453	0.272	0.132	0.104	0.028	0.011	0.000
Cumulative	0.453	0.724	0.856	0.960	0.989	1.000	1.000

Fig. 4 shows the loading plot for the first two components. In this simulation Tgmidpoint and Tgonset2 have a large positive loading on the first component, these components measure the level of adulteration in honey. The loading plot of Tgonset2 and Tgmidpoint were obtained from the Table 3. The first principal component (PC1) accounts for 45.3% of total variance as shown in Table 3 and the variable that correlated the most with PC1 are Tgonset2 and Tgmidpoint that are 52.4% and 52.6% respectively as shown in Table 3. Protein and maltose have large negative loadings on second principal component (PC2) and positively loading on sucrose, these components primarily measure the adulterated honey.

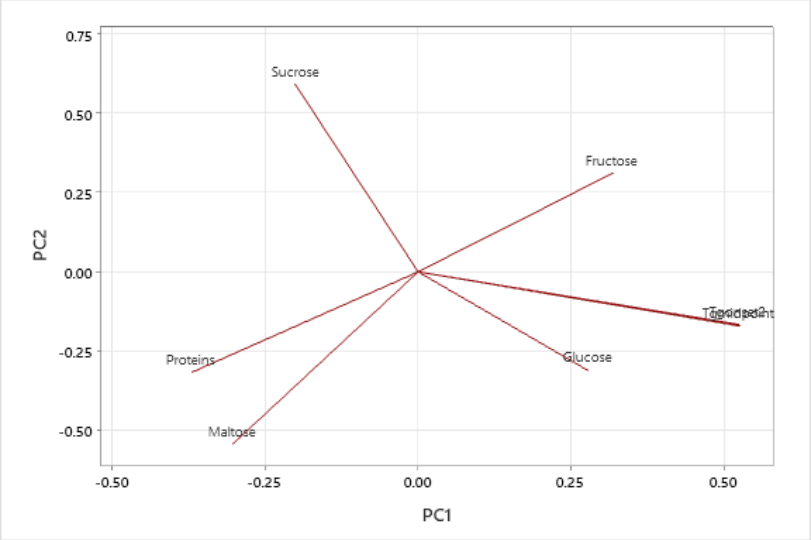

Fig. 4 - The loading plot of the variables which are the glass transition temperature and composition of sugar and protein

Table 3 - The eigenvectors value of the principal components

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Tgonset2	0.524	-0.166	-0.077	-0.237	0.379	-0.144	0.687
Tgmidpoint	0.526	-0.171	-0.065	-0.218	0.363	0.071	-0.711
Fructose	0.319	0.312	0.709	0.065	-0.186	-0.505	-0.069
Glucose	0.278	-0.312	-0.268	0.826	-0.106	-0.244	-0.026
Sucrose	-0.201	0.592	-0.385	0.091	0.487	-0.458	-0.081
Maltose	-0.304	-0.546	-0.109	-0.356	-0.126	-0.667	-0.105
Proteins	-0.370	-0.317	0.506	0.275	0.652	0.073	0.012

In this simulation plot, the adulteration level of the sample is grouped by circle. As shown in Fig. 5, most of the sample with 20% adulteration level (H80:20) were place at the outer layer of the circle and the lower the adulteration level which is 5% (H95:5) are in the inner circle that is near the pure honey. Only a few sample that are not in the right circle because every syrup has different composition of sugar and each of the composition effect the result in Tgonset2 and Tgmidpoint. Generally, as the adulteration increases, they move towards left quadrant except for agave syrup that move on the opposite quadrant due to the high composition of fructose. Rice and brown rice show the same behavior for the adulteration level based on the parameter analyzed.

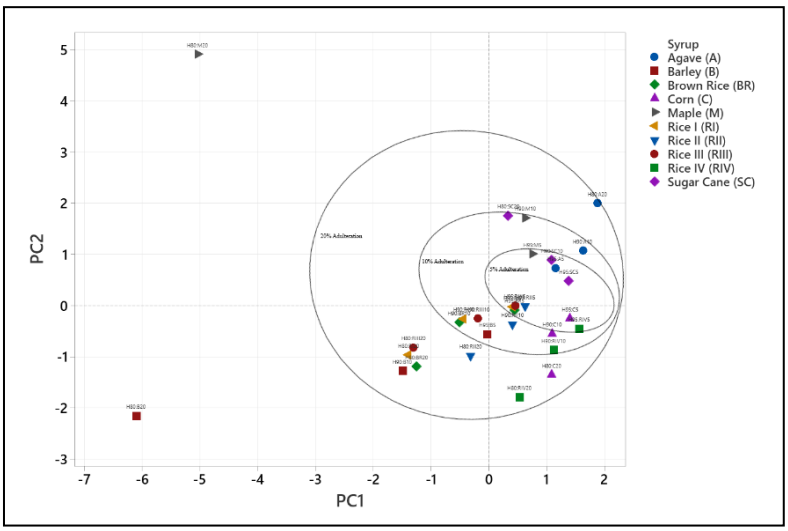

Fig. 5 - PCA score plot of the data from Table 1 using Minitab software

In Fig 6, the plot shows when pure honey is included. The position of the plot is different because when the data from pure honey is included, the calculation for eigenvector and eigenvalue is different. Based on this figure, the position of each sample can be analyzed. The sample move further away from the pure honey when the adulteration level increases.

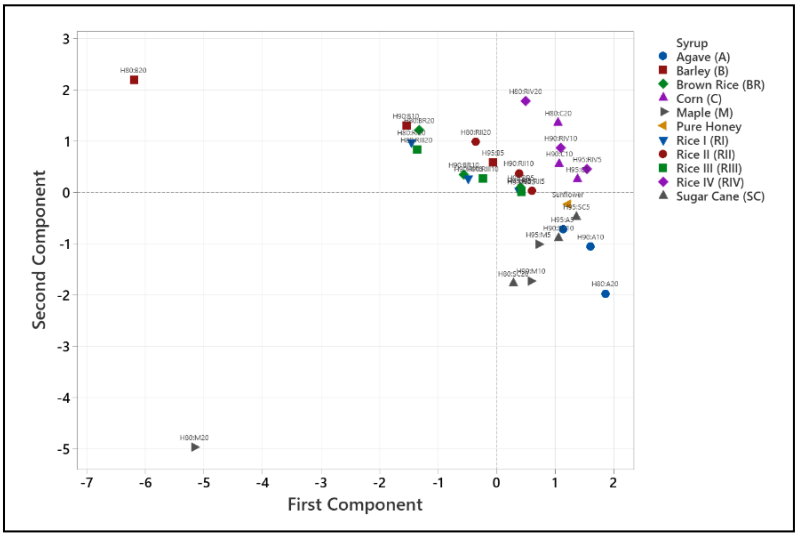

Fig. 6 - PCA score plot of the data with pure honey using Minitab software

In Fig. 7, the simulation output shows the biplot of the component using Minitab software. This biplot graph overlay the loading plot and score plot on the same graph. The result from biplot shows clearly why the sample behave in such way depending on the composition of their sugar. As shown in Fig. 6 and Fig. 7, there are two samples that are further from all other samples which are H80:M20 and H80:B20 samples. The 20% adulteration of maple syrup is more towards the second quadrant because of the high composition of sucrose and 20% adulteration of barley syrup has high composition of maltose. As in Fig. 5 maltose is the most negative correlated and sucrose is the most positive correlated in PC2 causes place out of the circle because they are highly marked.

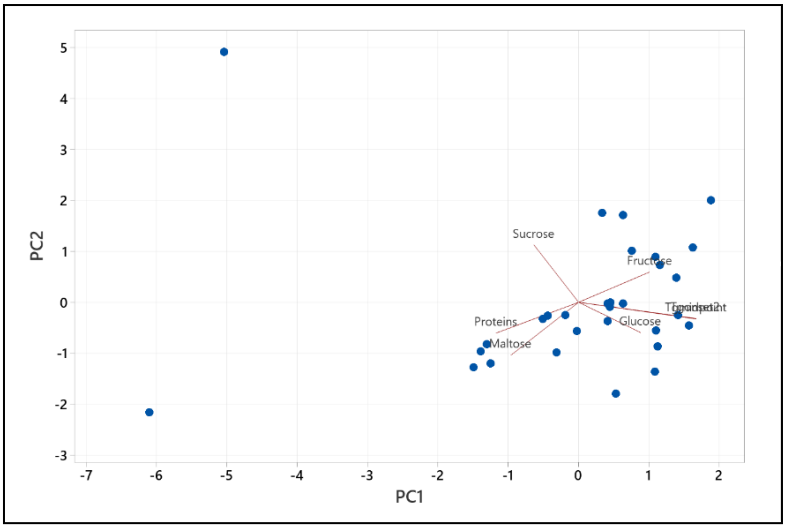

Fig. 7 - PCA biplot (loading and score) using Minitab software

Each of the adulterant has different composition of sugar and protein causes some behaviour different shown on the plot. However, in general, the pattern of the plot can be group based on the level of adulteration as circle on the Fig. 5 where barley and sugar cane showed the lowest effect compared to other samples where for sugar cane, the 5% and 10% adulteration are located inside 5% adulteration circle and for barley, the 5% adulteration is in 10% circle and 10% adulteration is in 20% circle. Each syrup has lower effect because of high in maltose composition for barley and high in sucrose for sugar cane cause the position of the sample in PCA plot is not in the right circle.

4. Conclusion

Nowadays there are many laboratories research has been done on how to detect honey adulteration of different type of adulterant. There are various methods have been studied to detect honey adulteration such as SCIRA, EN, ET, FTIR-ATR, DSC, NIRS, and LF1HNMR. Most of this method used multivariate data analysis to analyze the data obtained and the most used technique is PCA. PCA technique can be used to classify the data obtained from laboratory research and from this paper, PCA can classify the honey based on adulteration level with different type of adulterant. In this paper, 5% of adulteration cluster near the pure honey and as the level of adulteration increases, the samples move further from the pure honey. This technique can be applied to a random sample and the level of adulteration can be determined. Further study needs to be done for different type of honey for validation of this result.

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