Identification of Diastase Enzyme as An Indicator of Authenticity of Sumatran Forest Honey with Non-Destructive Method Using NIR Spectroscopy

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Abstract. The demand for honey as an immunomodulator increase during the Covid-19 pandemic. Many types of honey, especially Sumatran forest honey, sold in the market. However, circulating fake honey that is difficult to distinguish from real honey. It potentially harm the consumers.The main indicator to determining freshness and authenticity of honey is the presence of diastase enzymes. Near infrared spectroscopy (NIRS) technology can be used to identify the authenticity of honey based on the diastase enzymes.This study aims to develop an authenticity test of several types of Sumatran forest honey based on the level of diastase enzymes with non-destructive methods using NIRS with partial least square (PLS) analysis. The data from NIRS method will be correlated with honey chemical test data (diastase enzyme levels, hidroksimetilfurfural, moisture content, sucrose content, and acidity) according to SNI procedures. The result of this study was activity of the enzyme diastase can be detected using NIRS. NIRS spectrum obtained from standard diastase enzyme solution samples was relatively the same as 5 types of Sumatran forest honey samples.

INTRODUCTION

The Covid-19 pandemic encourages people to maintain their health. People need to consume food that have the ability as immunomodulators or immune enhancers [1]. Honey is a type of food that has long been popular with the properties of immunomodulators.

The increasing demand for honey by consumers gives honey farmers a great opportunity to reap greater profits. At the same time, the opportunity is used by irresponsible persons to act fraudulently. There are many counterfeits and removal of honey in order to get a double profit. Honey removal is usually done by mixing pure honey with a sugar solution be it sucrose, fructose syrup, or glucose [2]. While artificial or fake honey is usually made with a 100% sugar solution that is added citric acid so it is dangerous for people with sugar disease. The fake honey is then sold at a cheaper price, but some are just as expensive as real honey when the quality is much lower. The main indicator determining the freshness and authenticity of honey is the presence of diastase enzymes [3].

The enzyme diastase is derived from the body of bees that serves to convert polysaccharides into monosaccharides so that it can be classified as amylase [4]. The Badan Standardisasi Nasional Indonesia in 2018 set the level of diastase enzyme at least 1 DN (distase number) as an indicator of honey quality. While honey efficacious as an immunomodulator must have a minimum level of diastase enzyme 3 DN [1].

Destructive testing of diastase enzyme activity can use SNI 8664:2018 standard method on honey but it certainly takes a lot of time. A quick test of honey authenticity has been developed using non-destructive methods to obtain faster results but still accurate. The use of NIRS to predict the content of chemical compounds has been conducted since the 1980s ranging from water content test, protein, to fat.

A recent study from Aliaño-González [5] used NIRS to compare the color spectrum of real honey, rice sugar solution, invert sugar, cane sugar, and fructose syrup. Latorre [6] identified honey using NIRS based on the

geographical conditions of its nectar origin. While García-Alvarez [7] used NIRS to test the quality of honey from its macro compound content such as fructose, glucose, and moisture content. While Huang [8] used NIRS (visible near infrared spectroscopy) to predict DN enzyme diastase through vibrations and strains of O-H (6v), O-H (4v), and C-H (4v) in acacia honey, linen, and longan that have been through the heating process. The spectrum of waves used is between 400-1000 nm. The calibration models used are PLS (linear) and LS-SVM (non-linear) methods. The results of the study stated that there is a decrease in the level of the enzyme diastase in line with the increase in temperature in the process of heating honey. In addition, it is known that the activity of diastase enzymes in acacia honey and heated longans turned out to be higher than linen honey.

This study aims to develop an authenticity test of several types of Sumatran forest honey from Jambi and South Sumatera that circulating in Lampung market based on the level of enzymes distase with non-destructive methods using NIRS with partial least square analysis (PLS). This research is expected to be the first research in the development of portable tools for testing the authenticity of NIRS-based honey so that it can be used anywhere.

METHOD

This study used glassware, spectrophotometers, refractometers, vordex mixers, magnetic stirrers, pH meters, NIRS, and computers. The required ingredients include 5 types of Sumatran forest honey (each of them harvest at two times in different month), standart solution of diastase enzyme, iod solution, potassium iodide, acetic buffer, sodium chloride, aquades, starch, sodium bisuphite, potassium feroxide, zinc acetate, and sodium hydroxide. Honey samples were taken from forest honey producers in Jambi and South Sumatra. Honey was placed at room temperature without being given special treatment. Safe delivery of bottled honey samples using cardboard packaging.

The chemical analysis of honey in general was divided into 2 activities. *First*, testing samples of Sumatran forest honey chemically (destructively) include the activity of enzymes diastase, hydroxymetilfurfural, moisture content, and acidity based on SNI 8664:2018, as well as sucrose levels based on SNI 01-3545-2004 each three times the replay. *Second*, test with NIRS (non-destructive) with the NIR-Flex Spectrometer tool at wavelengths of 1000-2500 nm with a spectra resolution of 0.5 nm. Each sample was made three times a replay at three points. The recorded spectra data averaged into a data matrix (Dnxm) where m was the value of absorbance and n was the reference honey sample value of the destructive chemical test. Isometric tests were conducted using PLS on Unscrumble software version 10.1 to obtain calibration and validation values.

RESULT AND DISCUSSION

The results of the study were obtained in the form of NIRS data spectrum (Figure 1) from 5 samples of Sumatran forest honey with two series of harvesting and diastase enzyme standards each. The data was in the form of original spectrum that has not been done pretreatment.

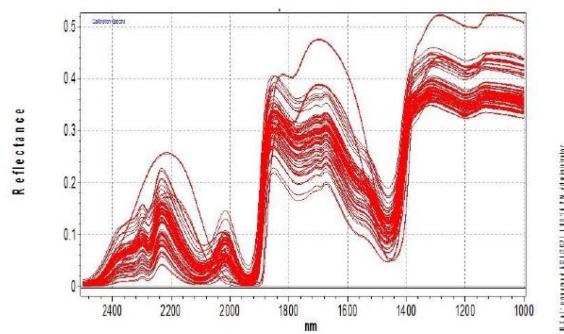

Figure 1. NIRS spectrum 5 samples of Sumatran forest honey and diastase enzyme standard

Figure 1 shows that the spectrum of standardized diastase enzymes and different types of Sumatran forest honey samples have almost the same pattern. This indicates that the enzyme diastase can be detected using NIRS. In addition, it was known that sumatran forest honey tested also contains the enzyme diastase. So, the honey was genuine and does not suffer damage due to excessive heating. The characteristic of real honey has a diastase enzyme content because the diastase enzyme is only produced by bees [4]. Huang [8] reported a decrease in diastase enzyme levels as the temperature in the honey heating process warmed up beyond 60°C for 2 hours. While the enzyme diastase is damaged at a temperature of >80°C.

Each peak and valley indicated by the NIRS spectrum indicates the presence of a specific chemical bond for which references are already available at each wavelength. The most distant curve of deviation was likely to be the curve of a standard solution of diastase enzymes whose moisture content was much higher than honey so nirs detects more H_2O contained in it.

Table 1. Results of destructive test of Sumatran forest honey

Sample	Moisture content (%)	Sucrose (%)	Acidity (NaOH 0,1N/kg)	Level of diastase enzyme (DN)	HMF (ppm)
SNI	Max. 22	Max. 5	Max. 50	Min. 1	Max. 40
A	30,00	25,30	47,35	2,15	39,10
B	30,25	22,65	45,65	2,79	279,00
C	19,20	1,36	34,45	2,74	1964,50
D	19,10	1,40	28,65	1,93	1317,50
E	19,35	32,75	27,35	3,43	1894,00

The destructive chemical test data (Table 1) obtained showed that the highest diastase enzyme is in honey E, namely bitter black honey. The value of its diastase enzyme >3 DN indicates that the honey qualifies as an immunomodulator. While other textured honey contains the enzyme diastase about 2 DN. This suggests that all honey samples are native and potentially immunomodulators.

Honey contains many simple sugars (monosaccharides) such as fructose and glucose resulting from the breakdown of sucrose which is a disaccharide by the enzyme invertase. Excessive levels of honey sucrose need to be suspected of the addition of other sugars to the composition of honey. Sucrose levels in honey can be affected by the presence of the enzyme invertase, an enzyme that converts sucrose into glucose and fructose. The optimum temperature of the enzyme invertase ranges from 30◦-50◦C (El sayed, 2015). Sucrose levels in room temperature honey are lower than cold temperature honey because the enzyme invertase is more active at room temperature than cold temperature.

The HMF analysis was conducted to determine the quality of honey affected by excessive heating, the addition of invert sugar and honey storage temperature. The quality of honey cannot be determined only by HMF analysis, but other analyses need to be done such as diastase enzyme activity, water content, reducing sugar, sucrose. HMF analysis can provide the information needed to estimate the total heat exposure of each type of honey. Includes heat used in processing, storage or shipping. Newly harvested honey already contains HMF and does not depend on the type of honey. The high value of HMF can be caused by damage that occurs from the honey heating process after harvesting or poor storage process (exposed to direct sunlight).

Honey water content is influenced by the humidity of the existing environment. This is because honey has hygroscopic properties, which is easy to absorb water. The higher the humidity of the environment, the higher the honey water content. If the humidity is 51%, the honey moisture content is 16.1%. If the humidity is 81%, the honey moisture content is 33.4% (Sarwono, 2007). The high water content of honey in Indonesia is caused by the relative humidity (Rh) of the air in Indonesia is high (Gojmerac, 1983). Indonesia's relative humidity (Rh) ranges from 60% to 90%, resulting in honey moisture content of about 18.3% to 33.1% (Sihombing, 2005). Low water content will keep honey from damage for a relatively long period of time. Prasetya and Andi (2014) explained that the high water content in honey will stimulate yeast activity to grow and develop in honey. The age of harvest also affects the composition of water in honey. Honey harvested in old age has less moisture content than honey harvested at a younger age. The longer the honey in the beehive, the evaporation of water content in the honey will be more perfect.

The acidity of honey is one of the parameters used to determine the quality of honey. This acidity level indicates the amount of free acid per kg of honey. In this study obtained acidity data for honey samples at cold temperatures and room temperature. In honey the room temperature has an average acidity of 45 ml NaOH 0.1N / kg and cold temperature honey the average acidity level is 32 ml NaOH 0.1N / kg.

The diastase enzyme is a leading indicator of honey authenticity, so diastase enzyme level data will be compared with NIRS data. The relationship between the results of destructive tests of diastase enzymes and nondestuctive (NIRS) sumatran forest honey is then tested by PCA method where the results are quite precise (Figure 2). From the results of the comparison obtained a pattern that can be used for databases and basic modeling of honey authenticity detection test tools.

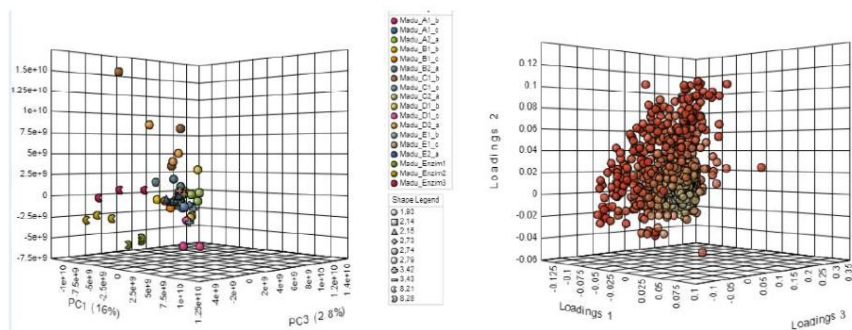

Figure 2. PCA test results between the destructive and nondestruktif aspects of Sumatran forest honey

CONCLUSION

O-H compounds that are thought to be components of sumatran forest honey diastase enzymes can be identified using NIRS at wavelengths of 1400 nm and 1900 nm. The results of the PCA analysis showed a database of points predicting the activity of the diastase enzyme. Further research is expected to result in a more complete database for the development of prototype honey authenticity test kits.

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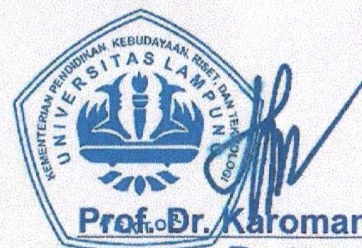

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