DIFFERENTIATION OF TEA LEAF PARTS (*CAMELLIA SINENSIS*) USING A NEAR INFRARED SPECTROSCOPY AND 2ND DERIVATIVE ANALYSIS

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ABSTRACT

Generally good quality of tea (*Camellia sinensis*) is taken from the tea buds that consist of flowery orange pecko (tip of the bud), orange pecko (second leaves) and, pecko (third leaves). In some cases stem are also included as a part of tea bud. Near infrared spectrum of each part of tea bud, including stem, were measured by the use FT-NIR spectrometer. Each part was dried up and grounded up into a powder and filtered using mesh #3. Each sample part was divided into 30 sub-part. Transreflectance spectrum of each sub-part was measured and averaged to increase s/n ratio of the spectrum. Second derivative were applied to all of the averaged transreflectance spectral to improve specral resolution and baseline corection. Four different pattern of second derivative signals were revealed, indicating that each part of the buds having different NIR characteristics.

Keyword: tea leaves, FT-NIRS, spectroscopy.

INTRODUCTION

Tea has become a global product with world consumption is quite high (Ditjen perkebunan, 2015). The quality tea it self is understood to have a wide range of quality ratings and therefore the price of tea varies greatly. The ranking of tea is usually done with evaluation techniques using sense of taste, touch and smell (Lawless, 1998). Therefore, sensing can be very subjective because it can be affected by physical and psychological conditions, so the determination of the quality of tea involves 3-5 experts who form a panel in which the decision was decided through an agreement based on the sensory experience of each member of the panel that consists of odd number of people. Such ranking of tea has lasted a long time and is often used on national tea market and even the world. Unfortunately, this technique can not be used at any time because of the need to collect a number of members of the expert panel in advance. To improve the consistency and accuracy of the rating, it would be required a quantitative method that allows the tea samples to be measured at any time and free of the psychic and phychological influences (Scott, 2009).

Near infrared spectroscopy has enabled solid sample to be measured non-destructively using trans-reflectance technique (Heise, 2001). Vibration energy of molecular bonding including O-H, N-H and C=O are key features of this kind of spectroscopy as their overtone and combination band absorption typically strong and fall in the range of 4000-8000 cm^{-1} (Ozaki, 2002). Since the overtones and combination band absorptions may appear very close or even overlap in that of spectral region then absorptions peaks are generally broad. In addition reflectance spectrum usually exhibit baseline shift and multiplicative due to the variation of sample size or technical setup. Unfortunately, broad spectral features and baseline shift substantially hampered a small variation among samples. Therefore baseline correction and improvisation spectral resolution are important for spectral differentiation.

This paper reports differentiation of tea bud parts (*Camellia sinensis*) namely flowery orange pecko, orange pecko, pecko and stem. Near infrared spectra of different parts of tea buds were measured and analysed by the use of 2nd derivative technique.

PROPERTY OF 1st AND 2nd DERIVATIVE SIGNALS

In general, the spectrum of transmission, absorption, reflection as well as trans reflection are experiencing baseline shift due to scattering. As a result

of this baseline shift, the relative height among the spectrum is difficult to be compared. Baseline shift can be corrected by performing the first derivative of the spectrum. Figure 1 (top panel) shows a simulation of two different spectra, i.e. a spectrum with a single Gausian function (blue) which has a peak at x = 20 and a spectrum of the superposition of two Gaussian amplitude contrast to the peak is at position x = 20 and x = 30 (red). Superposition spectrum of the two Gaussian has a higher baseline compared to the single Gaussian spectrum. The form the first derivative of the spectra is shown in Figure 1 (middle panel). It is clear that the first derivative spectrum directly diminish the baseline differences of the two spectra. Keep in mind that this spectrum derivation process will work best if the spectrum has good ratio of s/n. Because the derivative spectrum depicts the gradient curve at every point on the spectrum it self, the fluctuations due to noise will obtain significant amplification. Therefore the spectrum that contains noise needs smoothing before derivation process is done.
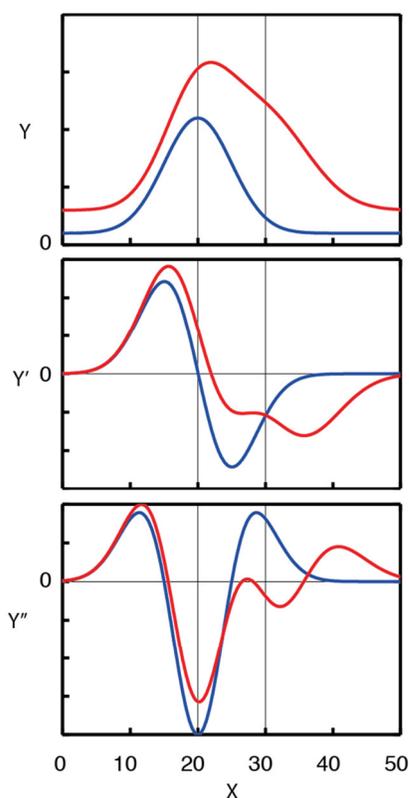
The second derivative spectra are shown in Figure 1 (bottom panel). The peak position (maxima) in the original spectrum is represented by the position of the valley (minimal) in second derivative spectrum. It looks clear that the resolution of the spectrum is increased in the second derivative spectrum.

The spectrum with superposition of two Gaussian (red) on the top panel shows that the second peak that should be at the right back (shoulder) that is not so clear, it becomes clear in the second derivative spectrum. It should be noted that the spectrum peaks in the second derivative has no particular meaning other than increasing the spectral resolution. So the second derivative spectrum is critical in identifying the peaks of the spectrum.

NEAR INFRARED ABSORPTION

The absorption of near infrared is generally associated with overtone and combination overtone of the molecular vibration energy related to oxygen and hydrogen bonding. Typical near infrared absorption on energy expanse 4000 to 10,000 cm^{-1} are shown in Table 1.

Table 1: The absorption of near infrared of some molecular bond (Stuart, 2004).

Wavenumber (cm^{-1})	Assigment
4545-4065	Combination C-H Stretching
5000-4545	Combination N-H dan O-H Stretching
6173-5556	Overtone C-H
7143-6250	Overtone N-H dan O-H
7682-8042	C-H Stretching
9091-8163	Second Overtone C-H
9804-9034	S-O Stretching
10536-9091	N-H dan O-H Stretching

Figure 1: Simulation of 1st (middle panel) and 2nd (bottom panel) derivative of single Gaussian curve (blue) and superposition of double Gaussian curves (red) centred at X=20 and=X-30 with different height (top panel).

EXPERIMENTAL

Sample preparation

The material used in this study is the fresh tea buds are picked from the tea plantation of Kemuning,

Karanganyar, Indonesia. After being picked the young tea leaves are sorted into four parts, as shown in Figure 2: flowery orange pecko, orange pecko, pecko and stem. The parts that have been sorted of tea are further dried in the sun for 1 hour with the aim of reducing the water content. When sun drying, the tea is dried in the traditional way that is fried using pottery where the temperature is kept constant at 60^0C. After the tea leaves are completely dry but not scorched, characterized by leaves which are easily crushed, the tea leaves are ground using a blender until the tea leaves become powder. Then the powder is sifted with mesh size no.3. Each kind of tea powder is stored in a different container, sealed to keep moisture from the air and is kept for 2 days. At this stage fermentation starts happening.

Figure 2: Tea bud (a) flowery orange pecko, (b) orange pecko, (c) pecko and (d) stem
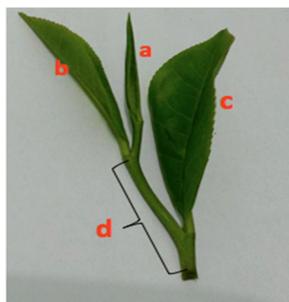
Near infrared spectral acquisition

Instrumen NIR spectrometer BUCHI NIRFlex SOLID N-500 were employed to measure transreflectance spectral in the region of 4000-8000 cm^{-1} with 4 cm^{-1}. Flowery orange pecko powder were devided into 30 parts and each part was mesured once. To improve s/n ratio the 30 spectral were then averaged. The process was repeated for orange pecko, pecko dan stem.

Data analysis

Transreflectan spectra of the four samples of tea that have been averaged lowered once to remove the baseline by first being smoothed using Savitsky-Golay technique with polynomial fit order 3at window length 11. The second derivative spectrum is done by being smoothed beforehand once again using the same techniques and parameters. The second derivative spectrum is aimed at improving spectral resolution which also increases the distinction power of spectrum among the samples. To distinguish the four second derivative spectra of the different parts of tea buds, every second derivative spectrum is to be subtracted with the second derivative spectrum of the stem.

RESULT AND DISCUSSION

Transreflectance spectra from four different sample namely flowery orange pecko, orange pecko,

pecko and stem in a region of 4000 – 7500 cm^{-1} are shown in Fig. 3. Broad spectral features and typical absorption of the O-H bonding vibration appears around 5227 cm^{-1}. Random baseline shifts and multiplicative effects are also present. Direct comparisons of those spectra still difficult since the baseline shifts and multiplicative effects distracted spectral interpretation.
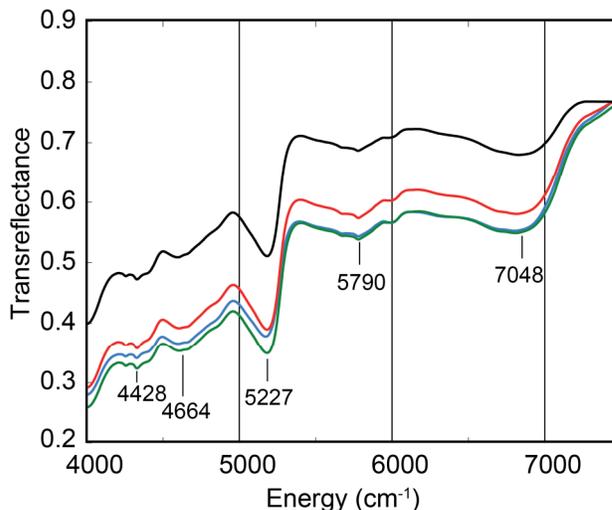

Figure 3: Transreflectance spectra of tea buds; flowery orange pekco (blue), orange pecko (green), pecko (red), and stem (black).
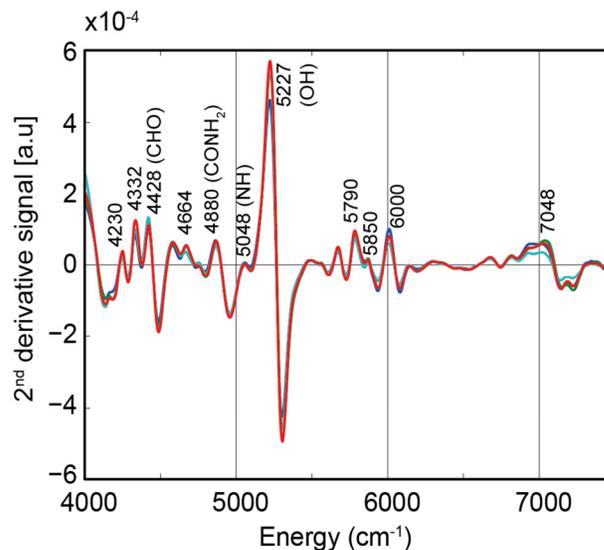

Figure 4: 2nd derivative spectra of tea buds for flowery orange pekco (blue), orange pecko (green), pecko (red), and stem (black). All wavenumber given on top of the peak are representing CH bonding, except are indicated different.

To overcome such disadvantages 2nd derivations were applied, resulting sharper structure spectra that are free from baseline shift as shown in Fig. 4. All spectra

clearly demonstrate similarity although small variations appear at around 7000 cm^{-1}. In 2nd derivative spectra, peaks indicating valley positions in transreflectance spectra.

No physical meaning given by valleys in 2nd derivative spectra as they are only mathematical implication of defining sharper peaks. To enhance spectral variations among those samples, the spectrum from stem was then used as a relative baseline, which means the remaining three spectra must be subtracted by the spectra from stem. The results are shown in Fig. 5. In this figure, subtle different of each spectrum relative to the spectrum of stem were magnified.
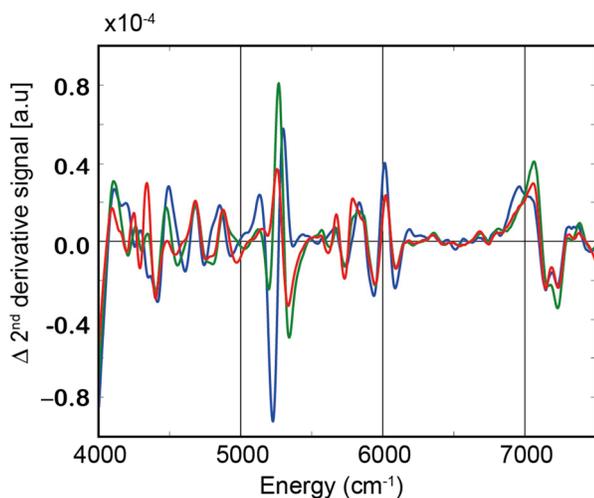

Figure 5. Difference 2nd derivative spectra of tea buds. Each of the 2nd derivative spectra was subtracted by 2nd derivative spectra of stem; lowery orange pekco (blue), orange pecko (green), pecko (red).

Signals in the positive side indicating absorptions in that particular wavenumber are larger than that of absorptions from the stem. Some peaks also broader as indicated by flowery orange pecko around 7000 cm^{-1} and 5100 cm^{-1}. Those broader peaks indicating that additional absorptions are present in that of particular sample that missing in stem. These unique features may be useful for marking or identification the uniqueness of certain property of flowery orange pecko, for example. This preliminary results need to be examined in more detail.

CONCLUSION

Near infrared transreflectance spectra of the tea buds reflect variations for flowery orange pecko, orange pecko, pecko and stem. Broad spectral features of the spectrum, baseline shifts and multiplicative effect may be avoid by employing 2nd derivative analyses. Since the spectrum of each parts of tea buds turned out to be quite similar then it is useful to compare all the spectra by the use of one reference spectrum as baseline. The reference spectrum may be taken from the set of the same spectra. It was found that flowery orang pecko exhibits unique absorption when compare to the other three parts of the tea buds although the origin the uniqueness need to further investigation.

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