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Application of NIR Spectral Absorbance Linearization and Gradient Shift in Quantifying Aqueous Glucose and Fructose Solution

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Spectral data analysis is an essential companion to spectroscopic experiment. The purpose is to produce a reliable data representation on examined samples, which in this research is on quantification of biochemical composition. Many different analyses have been introduced and performed regularly in spectroscopy measurement such as derivatives, principal component analysis, partial least square-discrimination analysis, normalization and linear regression. While in the other hand, innovative approach has been continuously introduced with improved solutions to current techniques. This paper introduced NIR spectral absorbance linearization and gradient shift in quantifying two aqueous monosaccharide solutions, namely glucose and fructose. In this paper, spectral absorbance linearization and gradient shift has manage to provide a better coefficient of determination, R^2 in quantifying glucose and fructose concentration (in unit of Brix) if compared to single wavelength analysis.

Key Words: Absorbance, Fructose, Glucose, NIR spectroscopy.

INTRODUCTION

Essentials of spectral manipulation: During spectroscopic measurement, spectrometers will produce the spectral signature of a measured sample, which in this work is two aqueous monosaccharide solutions (glucose and fructose), as the output. In the case of spectroscopic application in biochemistry, measured sample normally consists of a few molecular compounds which have tendency to absorb different wavelength of light at different capacity. This output is considered as raw and often undergo multiple mathematical, commonly statistical analysis before it is extracted as final data. The intention of this mathematical treatment to the original light spectrum is to find the possible linear relationship (R^2) between the absorbance and the expected biochemical composition and its magnitude in the examined material. This can be done at a single wavelength, several point of wavelengths or at a range of spectrum. The resultant computed coefficient of determination, R^2 of 1 is considered to be the perfect fit for linear measurement. Besides, root mean square error (RMSE) is often computed along side to the R^2. The raw spectrum retrieved from the spectrometer usually will be in the form of either reflectance or transmission value. However, when the analysis is performed, the raw optical data will be converted into absorption values. This is done because typical analysis and existing results on spectroscopy are presented as peak absorbance of a composition. In order to maximize correlation and repeatability of spectroscopic measurement, various mathematical analyses have been tested. This research applied spectral mathematical calculation as presented by Omar and MatJafri[1] in quantifying aqueous monosaccharide solution, namely glucose and fructose. In the experiment, 2 techniques were introduced in manipulating NIR spectral produced from spectroscopy experiment. These techniques are NIR spectral reflectance linearization and gradient shift which was implemented in monitoring apple and pear decay. The results obtained from these techniques were compared to analysis that makes use of single wavelength and it is proven that R^2 produced is much improved. For this paper, similar techniques are used to quantify aqueous monosaccharide solution and the results will also be compared to the analysis that make used of a single wavelength analysis. The purpose of this analysis is to reproduce analysis conducted by Omar and MatJafri on different biochemical composition with intention to produce an improved correlation between spectral data and biochemical composition, thus, resulting in better measurement accuracy.

Spectral data analysis: Mathematical treatment on raw spectral data is essential in producing optimum results for spectroscopic measurement. Various analyses have been performed

regularly in spectroscopy and proven its reliability while in the other hand, innovative approach has been introduced with improved solutions to current techniques. Derivatives are one of the frequently used methods in interpreting NIR spectrum for qualitative and quantitative analysis. This technique which is also known as derivative spectroscopy uses first or higher derivatives of absorbance with respect to wavelength. The first derivative will removes the baseline or offset produced by the scattering effects. On the other hand, the second derivative eliminates the gradient of the spectrum[2,3]. For example, Temma *et al.*[4] applying second derivative spectra in the measurement of sugar content in apple and apple juice. The purpose is to split any overlapping absorption peaks thus, the selection of appropriate peak for measurement and analysis can be done. The disadvantage of this technique is its amplification of spectral curvature. This condition often acceptable, but it may also raise the random noise to the spectrum. This may signify the problem as soon as the spectrophotometer reached its detection limit. The increase in random noise non-etheless, can be reduced by smoothing the spectra before the differentiation can take place[3]. In an experiment conducted by Sambongi *et al.*[5], they have implemented spectral reflectance using normalization method in detecting early gastric and colon cancer. From their observation, the spectral reflectance of early cancer and normal tissue are similar at long wavelength and shows some feature for early cancer at short wavelength. This method can reduce the problems related to the change of distance and angle between endoscopy probe and tissue. Therefore, spectral reflectance $f(\lambda)$ is divided by the reflectance of a long wavelength $f(\lambda_L)$ (*i.e.*, $f(\lambda)/f(\lambda_L)$ where $\lambda_L = 640$ nm.

The application of spectroscopy in agriculture industry, commonly, the mathematical analysis performed on the spectrum is intended to identify the magnitude of certain biochemical composition that is related to quality specification of agriculture produce such as fruits. For instant, as broadly been discussed through earlier chapter, the intrinsic quality parameters of fruits are sugar and acid content as well as firmness. However, there are also mathematical analyses which have been conducted by various researchers to distinguish fruits variety. Abu-Khalaf *et al.*[6] have used multivariate data analysis on NIR spectra ranging from 700-1100 nm to distinguishing carrot's characteristics. Multivariate data analysis is performed to many different variables simultaneously and it offers various methods for efficient simplification. This method reveals the main structures and relationships in large data tables. In addition, it also gives maximum information and minimal repetition and noise through relatively simple output graphs and tables. Abu-Khalaf *et al.*[6] have used two multivariate analyses which are principal component analysis (PCA) and partial least square-discrimination analysis (PLS-DA) in distinguishing carrot's characteristics through NIR. Principal component analysis can be used to identify patterns in a data set derived from recording several characteristics at a time. This method is able to eliminate redundancy in univariate analyses. Partial least square-discrimination analysis, in the other hand, as a supervised classification method, was used for classifying carrot samples according to their cultivation method and cultivar[6]. In a related example, Huang *et al.*[7] have used partial component analysis to discriminate juicy peach varieties by VIS/NIR spectroscopy ranging from 401-1000 nm.

Aqueous glucose and fructose spectroscopic identity: Glucose and fructose are carbohydrate and have the same molecular formula, $C_6H_{12}O_6$, but with different structures. Glucose and fructose are categorized differently as carbohydrate derivatives. Glucose is classified as an aldehyde while fructose as a ketone[8]. Glucose and fructose (monosaccharide) are common carbohydrate available in fruits besides sucrose (disaccharide) and starch (polysaccharide) and its notable earliest determination was conducted by Widdowson and McCance[9] through titration method. The status of water state in food products has always been investigated and continuous research in this field has led the development of new details supplementing earlier works on water behaviour in food such as carbohydrate water solutions[10]. Water is the largest component of most fruit and vegetables. Therefore, NIR spectra of samples with high water contents, above 80 %, are strongly dominated by spectral signature from water[11,12]. NIR spectroscopy has been extensively used to study water and water-solute interactions due to the low absorptivity of water in NIR region hence permits higher pathlength than other techniques[13]. The spectra which are dominated by the water spectrum will have peak absorption at 760, 970, 1190, 1450 and 1940 nm[12]. In this study, glucose and fructose concentration is represented in unit of Brix. In fruits, Brix is actually the summation of the pounds of sucrose, glucose, fructose, vitamins, minerals, amino acids, proteins, hormones and other soluble solids over one hundred pound of the particular sample[14]. However, the composition of fruits juice is almost entirely sugar thus the Brix should be almost equivalent to the actual sugar concentration[15]. In this paper, Brix represent the percentage of sugar (glucose/fructose) available in the sugar-water solution.

Spectroscopic empirical setup: In this research, the spectroscopic instrumentations used are from ocean optics and the setup is illustrated in Fig. 1. The ambient temperature throughout the experiment was measured at 25 ºC. The sugar samples used are prepared by diluting glucose (Unilab Chemicals) and fructose (HmbG Chemicals), in powder form, using pure water (reverse osmosis) and were calibrated using PAL-3 Atago refractometer. Brix is used as the measurement unit since based on literatures, most of related researches is using Brix in quantifying sugar concentration in fruits. In this experiment, the response obtained was only due to mixture between water with glucose or fructose. This is done in order to retrieve direct relationship between optical parameters with the sugar concentration in water. The concentrations of sugar prepared are between 0-30 Brix, following the range of common concentration of sugar in fruits. After the samples have been prepared and the quartz cuvette has been placed on the cuvette holder, a black anodized aluminum cover was used to cover the cuvette in order to eliminate ambient light and to block the light path when taking dark current measurements.

The spectral waveform was measured using Jaz spectrometer (spectral response: 650-1100 nm) that uses Sony ILX511B linear silicon CCD array detector with sensitivity of up to 75 photons/count at 400 nm and 41 photons/count at 600 nm. For spectral smoothing and intensity control purposes,

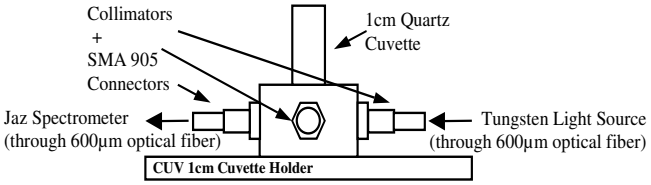

Fig. 1. Experimental setup for glucose and fructose absorbance measurement

some custom setup to the retrieved raw waveform can be adjusted. The parameters that have been set prior to the experiment includes integration time = 3 ms, spectra averaged = 5 and boxcar smoothing = 1. Light source used was tungsten halogen lamp with spectral wavelength between 360-2000 nm and colour temperature of 2960 K. The y-axis of the graph is represented in the unit of counts for its intensity. Counts are the raw output data produced by the analogue to digital converter of the spectrometer. SpectraSuite software allows this measurement to be converted into absorbance in the unit of OD (optical density). The software uses this equation to evaluate each pixel on the detector and produce the absorbance spectrum[16]:

$$A_\lambda = -\log_{10}\left(\frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda}\right)$$

where: A = absorbance at wavelength λ, S = sample intensity at wavelength λ, D = dark intensity at wavelength λ, R = reference intensity at wavelength λ (measured through empty cuvette).

The range of NIR spectral absorbance used to measure glucose and fructose concentration was retrieved between 960 and 1000 nm. This range was identified to produce the best results for the current analysis. Furthermore, one of the peak absorbance for water is at 970 nm, lies within this range. The NIR spectral absorbance for every sugar concentration was generated to retrieve its linear equation and coefficient of determination, R^2. Two analyses have been performed in this research. The first analysis is the quantification of sugar concentration through the changes of NIR spectral gradient. It was observed that from 32 absorbance spectra, for samples with sugar concentration between 0-30 Brix, lower NIR spectral gradient is produced for higher sugar concentration. This observation was then quantified by generating R^2 between the spectral gradient and sugar concentration. The second analysis is in the measurement of sugar concentration through spectral absorbance linearization. The value of R^2 between absorbance and sugar concentration was observed to be lower for higher concentration of sugar, indicating that there is an improvement in spectral linearity. This observation was then quantified to attain the value of spectral linearization that is presented by each spectrum R^2 against the sugar concentration. Fig. 2 shows the linear equation and R^2 generated from absorbance spectrum of glucose concentrations and illustration on concept of spectral linearization and gradient shift. On the other hand, Fig. 3 shows the linear equation and R^2 generated from absorbance spectrum of fructose concentrations. All regression process in this paper was simulated using MS Excel.

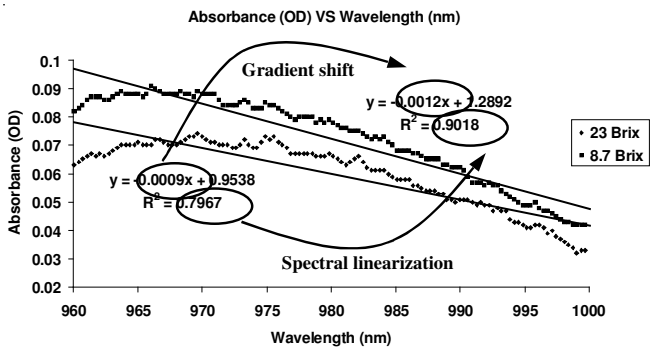

Fig. 2. Absorbance spectra for 8.7 and 23 Brix of glucose concentration

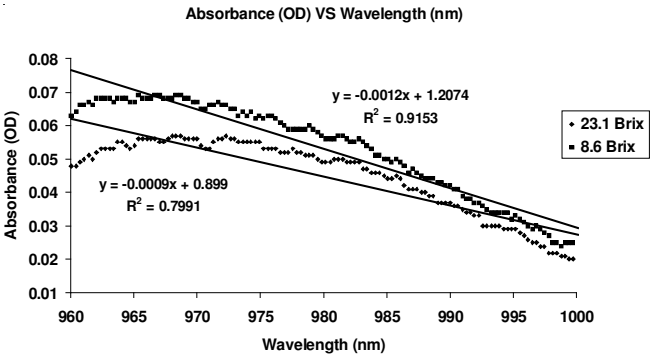

Fig. 3. Absorbance spectra for 8.6 and 23.1 Brix of fructose concentration

RESULTS AND DISCUSSION

The degree of growth in linear relationship between absorbance and sugar concentration was determined by presenting the findings alongside the conventional single wavelength measurement of sugar concentration. Frequently, the range of wavelength used for the measurement of organic compound is between 1000-2500 nm. The lower range of NIR between 700-1000 nm is less considered if compared to other NIR range since its absorptivity is less significant. However, this paper intended to enhance the usefulness of the entire NIR wavelengths and since common single detector such as photodiode in the market is having peak response within this range of wavelengths. The application of photodiode in quantifying a specific organic compound may eliminate the use of complex spectroscopic instrumentation.

Since the sugar sample examined in this experiment is in the form of aqueous solution, therefore the entire NIR waveform obtained may have high influence from water. This is the reason why during the measurement, the peak absorbance was detected at two points which is at 760 and 970 nm which are the peaks associated with water. However, wavelength at 760 nm fail to generate any relationship in quantifying sugar concentration since the absorbance value is too low. Noteworthy range of wavelength is acknowledged within 940-985 nm. Therefore, the linear equation and R^2 was generated for every 5 nm for the stated range of wavelengths. Table-1 summarizes the findings which show the linear relationship between absorbance (in optical density) with sugar (glucose and fructose) concentration. The best linearity was recorded at wavelength 954.80 and 965.05 nm for glucose and 965.05 nm for fructose.

TABLE-1
SUMMARY OF THE LINEAR RELATIONSHIP BETWEEN ABSORBANCE AND SUGAR (GLUCOSE AND FRUCTOSE) CONCENTRATIONS

Glucose			Fructose		
λ (nm)	Linear equation	R^2	λ (nm)	Linear equation	R^2
940.18	y = -0.0004x + 0.0114	0.7467	940.18	y = -0.0005x + 0.0029	0.6658
945.18	y = -0.0005x + 0.0238	0.8247	945.18	y = -0.0005x + 0.0122	0.7555
950.18	y = -0.0007x + 0.0449	0.9016	950.18	y = -0.0008x + 0.0336	0.8799
954.80	y = -0.001x + 0.0714	0.9208	954.80	y = -0.001x + 0.0559	0.9161
960.10	y = -0.001x + 0.0897	0.9203	960.10	y = -0.001x + 0.0744	0.9192
965.05	y = -0.0009x + 0.0956	0.9206	965.05	y = -0.0009x + 0.0782	0.9338
969.99	y = -0.0007x + 0.0927	0.8863	969.99	y = -0.0008x + 0.0765	0.8981
974.92	y = -0.0006x + 0.0895	0.8622	974.92	y = -0.0006x + 0.0706	0.8293
980.50	y = -0.0004x + 0.0798	0.7589	980.50	y = -0.0005x + 0.0626	0.7865
985.08	y = -0.0004x + 0.0712	0.6821	985.08	y = -0.0004x + 0.055	0.6233

Spectral absorbance linearization: In this section, the relationship between spectral linearity and sugar concentration is regressed as linear and quadratic equation. The result for linear regression is shown in Fig. 4. R^2 obtained is 0.9369 for glucose and 0.9025 for fructose. R^2 generated for glucose is higher than peak value stated in Table-1. However, lower coefficient recorded for fructose. The same graph has been resimulated to generate quadratic regression as shown in Fig. 5. This is done due to the visible observation that the data plotted on the graph appear to have a curvature pattern. The correlation has shown a further improvement with R^2 for glucose is 0.9834 and for fructose is 0.9799. The result has shown that the application of spectral absorbance linearization can improve prediction of sugar concentration thus may provides better measurement accuracy in quantifying glucose and fructose concentration.

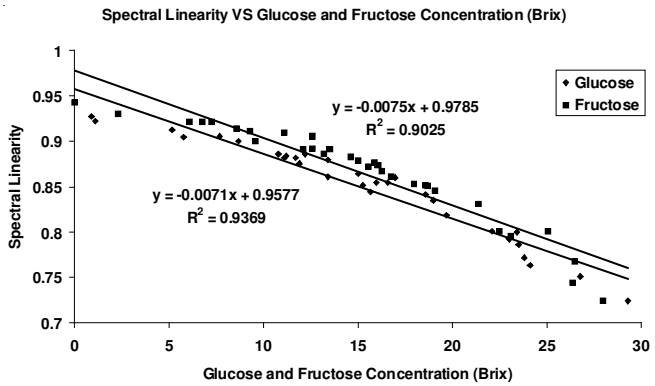

Fig. 4. Linear regression between spectral linearity and glucose/fructose concentration for λ = 960-1000 nm

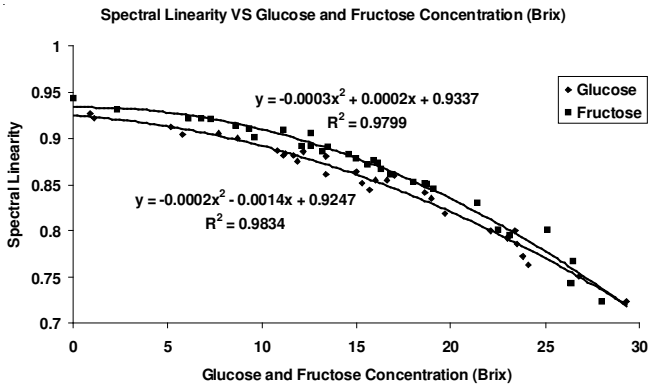

Fig. 5. Quadratic regression between spectral linearity and glucose/fructose concentration for λ = 960-1000 nm

Spectral absorbance gradient shift: In this section, the relationship between spectral gradient and sugar concentration is regressed as linear equation and the result is shown in Fig. 6. The R^2 generated a better value than those stated in Table-1 with R^2 for glucose is 0.9834 and for fructose is 0.9799. However, since the gradient of the spectra obtained from glucose and fructose is very low and does not deviate much between high and low sugar concentrations, therefore the quantification process losses its resolution. Despite the lower resolution shown in this experiment for spectral gradient shift, experiment conducted by Omar and MatJafri[1] does show a good resolution, comparable to the single wavelength analysis, but with better coefficient of determination.

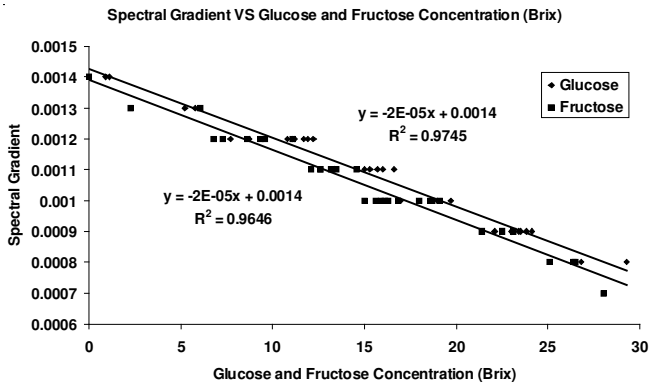

Fig. 6. Linear regression between spectral gradient and glucose/fructose concentration for λ = 960-1000 nm

Conclusion

This study has reapplied spectral analyses which were introduced by Omar and MatJafri[1]. Spectral absorbance linearization and gradient shift applied in this paper were implemented to quantify aqueous glucose and fructose solution using NIR range of wavelength between 960-1000 nm. Both techniques have managed to improve the coefficient of determination, R^2 in quantifying glucose and fructose concentration in the unit of Brix. However, despite able to produce a better relationship with sugar concentration, spectral gradient shift for this experiment has shown a weak resolution in representing sugar concentration. Further experiment and analysis will be continuously conducted using these analyses for different samples to test on its stability, repeatability and reproducibility in quantifying biochemical composition.

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