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FEASIBILITY OF NEAR-INFRARED REFLECTANCE SPECTROSCOPY FOR PREDICTING AMINO ACIDS COMPOSITION IN EDIBLE BIRD'S NEST

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Abstract. A preliminary study of near-infrared reflectance spectroscopy (NIRS) for the feasibility of analytical monitoring of amino acids and total protein compositions in edible bird's nest (EBN) was conducted. The training (n=134) and validation sets to develop the equations were built with local unprocessed EBN samples sourced from different states of Malaysia. The regression method employed was modified partial least-squares (MPLS). The values of standard error for cross validation (SECV) and the coefficient of determination (r^2) of the calibrations of these constituents for use to predict of amino acids in EBN were determined, but with a low predictive ability. To find an acceptable accuracy for each constituent is to increase the number of training samples. The findings, however, showed a potential alternative for the implementation of near-infrared reflectance spectroscopy technology in this field of analysis.

Keywords: edible bird's nest (EBN), near-infrared reflectance spectroscopy (NIRS), amino acids composition

INTRODUCTION

Edible bird's nest (EBN) refers to the nest produced by several different swiftlet species. The nests are constructed from the saliva of swiftlets, which has been secreted from the pair of sublingual salivary gland of swiftlets during nesting and breeding season. EBN consists of high valued glycoprotein rich with amino acids, carbohydrate, calcium, sodium and potassium (Norhayati *et al.*,2010). Near-infrared reflectance spectroscopy technique has extensive application for the analysis of constituents of agricultural crops, feeds, and food (Roberts *et al.*, 2004). At present, there is a dearth of published data on this subject matter; this preliminary study therefore was to explore the possibility of near-infrared reflectance spectroscopy analysis to predict the value of amino acids composition and total protein of the EBN sample matrix .

MATERIALS AND METHODS

EBN Samples

A total number of 134 commercial unprocessed EBN samples were received throughout the year for routine analysis of amino acids and other parameters by this laboratory. The sources of these samples were mostly from the southern zone of the Peninsular Malaysia. Each remaining sample was crushed into fine grains using hand mortar, kept in 50 mL plastic test tube each, capped and stored at 4 °C storage prior to near-infrared reflectance spectroscopy analysis.

Reference Analysis

(i) Protein analysis was performed according to Kjeldahl method, using 6.25 as a conversion factor (AOAC method, 1995). Briefly, finely grounded EBN sample (0.1 g) was digested with 12 ml concentrated sulphuric acid in the presence of a catalyst (3.5 g potassium sulphate, 0.4 g copper sulphate) to convert sample nitrogen to ammonium sulphate. The acid solution was made alkaline with 40% sodium hydroxide solution. The ammonia was distilled and collected in an excess of 4% boric acid solution followed by titration with 0.2N sulphuric acid solution.

(ii) The 18 amino acids of the samples were analyzed based on Waters method of analysis (Waters AccQ-Tag Chemistry Package Instruction Manual) using Waters Aquity H-Class UPLC System with photodiode/fluorescent detector. The amino acids determined include: histidine, arginine, threonin, valine, methionine, isoleusine, leusine, phenylalanine, tryptophan, lysine, aspartic, glutamic, serine, glysine, alanine, proline, tyrosine, cystine. Total amino acids were extracted by acid hydrolysis while tryptophan was extracted by alkaline hydrolysis.

Near-Infrared Reflectance Spectroscopy Analysis

A FOSS NIRSystems model 5000 (Silver Spring, USA) was used to measure reflectance spectra of the same set of samples from 1100 to 2500 nm. A standard ring cup with 4.7 cm diameter was used for the measurement. The reflectance spectra were collected in duplicates. Calibrations were developed using WinISI II software version 1.5e. Near-infrared reflectance spectroscopy equations for the parameters under study were obtained using modified partial least squares (MPLS) regression. Outliers were removed for selection of calibration and validation sets. Predicted results were summarized as the standard error of cross validation (SECV).

RESULTS AND DISCUSSION

In this study, several statistics of interest could be obtained. It is intended that the standard error of calibration (SEC) needs to be as small as possible. The second statistic of interest is the coefficient of determination (r^2) between the spectra

Table 1. Cross validation of amino acid composition and total protein of EBN samples

Constituent	N	Mean	SEC	SECV	RSQ	1-VR
Total Protein	101	53.8277	0.4262	1.1715	0.8543	0.362
Proline	107	3.0185	0.2888	0.6177	0.8235	0.233
Aspartic acid	104	3.6459	0.3028	0.6001	0.8207	0.291
Glutamic acid	107	2.7825	0.2076	0.4052	0.8327	0.220
Serine	98	4.0711	0.2819	0.6486	0.8485	0.285
Glycine	90	1.2858	0.1347	0.4298	0.9109	0.365
Histidine	103	1.4046	0.1071	0.2447	0.8203	0.228
Arginine	105	4.5075	0.4965	1.15021	0.8578	0.474
Threonine	105	2.4639	0.2255	0.4968	0.8328	0.307
Alanine	103	1.1527	0.0904	0.2017	0.8109	0.203
Tyrosine	98	2.5676	0.2464	0.5119	0.7447	0.264
Valine	107	1.4756	0.1534	0.3184	0.8100	0.337
Methionine	120	0.3795	0.1176	0.2115	0.8433	0.568
Cystine	107	1.0283	0.2133	0.3939	0.8676	0.631
Isoleusine	106	0.6043	0.0633	0.1275	0.8229	0.394
Leusine	103	2.4445	0.2130	0.4678	0.8392	0.431
Phenylalanine	104	2.1802	0.1458	0.3904	0.8937	0.405
Tryptopan	114	0.6542	0.0791	0.1339	0.8403	0.547
Lysine	103	1.1455	0.1294	0.3011	0.8384	0.421

SEC = Standard error of calibration; SECV = Standard error of cross validation; RSQ = R square; 1-VR = 1-variance ratio

and the analytical values, whose values range from 0 to 1. A value of 1.0 indicates that all of the spectral differences between samples correspond perfectly with differences in analytical values. From this study, r^2 values obtained for each constituent ranged between 0.74 to 0.91 (Table 1). The third statistic of interest is the proportion of variation explained by cross validation, "1-VR" (variance ratio) or the coefficient of determination of cross validation. This should be close to 1 and similar to the r^2 of calibration. Therefore 1-VR is the coefficient of determination (r^2) between the laboratory values and the predictions made during cross validation. The fourth statistic is the standard error of cross validation (SECV) that needs to be low values, similar to the standard error of calibration. Based on these statistics, the calibration equations obtained present a low predictive ability for the majority of constituents. According to their 1-VR values (<0.90), the equations for these analyses need to be further improved for quality assurance purposes.

One of the important parameters to be considered, beside homogeneity, is the

moisture content. The major components of food (water, protein, carbohydrates, and lipids) contain the overtones and combination of these molecules' fundamental vibrations particularly those involving hydrogen (Osborne, 1993). It is not sure whether a significant difference of moisture contents between each EBN's sample could give rise to a certain degree of inaccuracy between laboratory and near-infrared reflectance spectroscopy results. A different set of EBN samples (n=40) was tested for moisture content. The results obtained were between 8.73-18.63%, with an average of 13.68 % ± 4.55. The tolerance for moisture content according to Malaysian standard (MS2334:2011) is <15%.

CONCLUSION

In this feasibility study, the results demonstrate the capability of near-infrared reflectance spectroscopy technology to analyze important amino acids and total protein of unprocessed EBN samples. To increase the predictive ability more training samples need to be introduced in order to strengthen the quality of these equations. Currently, there is not much published data by other authors for the purpose of comparison, as well as the scarcity of EBN samples, the accuracy of the equations obtained in this study could not be confirmed.

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