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Prediction of starch content in meatballs using near infrared spectroscopy (NIRS)

[1*]Vichasilp, C. and [2]Kawano, S.

[1]*Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon 47160, Thailand*

[2]*Environmental Sciences and Technology, Faculty of Agriculture, Kagoshima University, Kagoshima city 890-0065, Japan*

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Abstract

Meatballs are a popular food in Asian countries. A good quality consists of low starch. In this study, the quality of meatballs was evaluated by starch content using short and long-wavelength near infrared spectroscopy (NIRS). The result found that long-wavelength NIRS can predict starch contents in all kinds of meatballs. The model of beef meatballs showed a high coefficient of multiple determination of validation set *(R^2-val)* of 0.97 and a low standard error of cross-validation *(SECV)* of 2.64%; the chicken meatballs model had an *R^2-val* of 0.97 and a *SECV* of 2.63%; and the pork meatballs model had an *R^2-val* of 0.98 and a *SECV* of 2.37%. In addition, a universal model was created by combining the spectra of all meatballs. The universal model had an coefficient of multiple determination of calibration set *(R^2-cal)* of 0.98, standard error of calibration *(SEC)* of 2.22%, *R^2-val* of 0.97, standard error of prediction *(SEP)* of 2.67% and Bias of 0.05%. The results indicated that NIRS can predict starch contents with high accuracy and could apply for quality classification via rapid analysis.

Introduction

Meatballs are a popular food in Asian countries such as Japan, Indonesia, Malaysia and Thailand. Nowadays, there are several kinds of meatball available on the market, such as beef, pork and chicken. To make meatballs, fresh meat is crushed into minced meat and mixed with flour, salt, and food seasonings, such as garlic and pepper. Then, the mixed ingredient meat is formed into a ball shape by human hands or by machine. The gel strength of the meat is set in warm water (50^{o}C), cooked in hot water (95^{o}C), and allowed to cool down before packing for sale.

Commercially, meatballs are graded by the percentage of starch in the formula. However, due to advancements in food technology, high quality meatballs can be imitated, using high amounts of starch, low amounts of meat, and the addition of food additives to improve physical and sensory properties. Some meatball products are made with starch levels of more than 50 percentage (w/w) and are sold as high quality meatballs for a higher profit. Considering this, it is difficult to grade meatball quality by visible and sensory test evaluation. A chemical analysis of starch content such as differential scanning calorimetric, iodine staining, and enzyme techniques is necessary for meatball classification. However, these methods are costly, laborious and time consuming work. A quick, accurate, low cost method with a smaller sample preparation is needed.

Near infrared spectroscopy (NIRS) is spectroscopic technique using near infrared region ranging from 700 nm to 2500 nm. The near infrared light stimulates vibrations of outer electron of compounds that have C-H, N-H, O-H and S-H groups. The light absorption of each compound has a specific absorbance pattern that makes NIRS to recognize and identify the chemicals. NIRS was wildly applied for quality identification of various agricultural products (Wu *et al*., 2008; Kapper *et al.*, 2012; De Marchi, 2013; Mariani *et al*., 2014; Travers *et al*., 2014; Huang *et al*., 2014; Prevolnik *et al.*, 2014). In this study, the feasibility of NIRS was used to investigate the starch content in meatballs. The finding will be beneficial for classification of meatballs quality and could apply for commercial use via rapid analysis.

Materials and Methods

Preparation of meatballs

The meatballs were made from three different kinds of meat material (beef, pork and chicken). To produce meatballs, fresh meat (500 g) was minced

*Corresponding author.
Email: *c.vichasilp@gmail.com*
Tel: +66-42-771-460; Fax: +66-42-771-460

for 5 minutes in a mixing machine (Tiger SKF-B, Osaka, Japan) and then mixed with different percentages of corn starch per fresh meat (0, 10, 20, 30, 40, and 50%). After the meats and starch were mixed together, other ingredients, such as salt (3%), pepper (0.4%), baking soda (0.2%) and cool water (20%) were added and mixed in the machine for 3 minutes. The mixed ingredient meat obtained was shaped into small circular figures by hands (as per traditional meatball processing) with average size of 1 inches diameter and suddenly dropped into warm water (50°C) for 20 minutes for gel strength setting. Then, the raw meatballs were cooked in boiling water (90°C for 20 minutes), kept in cold water (5°C for 15 minutes), and dried in open air (10 minutes). For each meatball material source, ten samples were made for each formula (a total of 180 samples). Three replicated NIR spectra were collected for each sample, and the average value was used for model calibration.

Spectral acquisition

The room temperature of laboratory was set at 25°C during the experiment period. Prior to spectral acquisition, each meatball was cut down the middle into two pieces by knife. The cut ball was incubated in a water bath covered with a thin polyethylene film at 25°C for 30 minutes. The smooth surface of the meatball was used to contact the NIRS acquisition part.

Short-wavelength NIRS

The spectra at a short-wavelength region (700-1100 nm) were collected by using a portable FQA-NIRGUN instrument with interactance mode (Shibuya Seiki Co. LTD., Shizuoka, Japan). An opal glass plate was used as reference material. The average scan was set at 32 scans with the time per single scan set at 100 ms integration time. The obtained spectrum data was converted to ASCII format before regression model calibration.

Long-wavelength NIRS

The NIR spectra were acquired by using a Buchi NIRFlex N-500 instrument (Buchi, Switzerland) equipped with NIRFlex Solids with a reflectance mode at a long-wavelength NIRS (1000-2500 nm) with 0.4 nm interval recording. The parameters of NIR acquisition were optimized at 32 scans on average (time per single scan of 500 ms integration time). A spectralon (Labsphere, North Sutton, New Hampshire, USA) was used as reference material prior to spectra recording. Before scanning NIR spectra of the next sample, the NIRS acquisition glass was cleaned with alcohol to eliminate moisture and fat.

Data analysis

The NIR spectra and value of starch content in percentage of each meatball formula were imported to Unscrambler software (version 9.8, GAMO, Oslo, Norway). Mathematical pre-treatments such as Savitzky-Golay (S. Golay) smoothing and 2nd derivative transformation were applied to remove noise and baseline shifts from a scattering effect. The partial least square (PLS) regression model was calculated with "leave-one-out" cross validation to prevent over fitting of the calibration model.

Wavelength range optimization

The wavelength range was optimized as per the following; the lowest wavelength was fixed constantly and the longer wavelength was changed respectively to obtain the best statistical results (1000-2500, 1000-2300, 1000-2100 and 1000-1900 nm). Further, the longer was adjusted at the fixed short wavelength from previous results. The wavelength showing the best statistical results was used to establish a PLS model.

Universal model

The spectra of each kind of meatball (beef, pork and chicken meat) were combined together. The NIR spectra and the value of starch content in meatballs were imported to Unscrambler software for calculation. Spectra treatments such as smoothing (S. Golay) and 2nd derivative transformation were applied. The PLS universal model was built from the spectral data and the starch content and a calibration set and validation set were used to prove accuracy.

Statistical analysis

The coefficient of multiple determinations *(R^2)* of calibration and validation *(R^2-cal, R^2-val)* was considered to prove the accuracy of the predictions as calculated with equation 1. The standard error of calibration *(SEC)* and prediction *(SEP)* were calculated following equation 2 and 3.

$$R^2 = 1 - \frac{\sum (y_{cal} - y_{act})^2}{\sum (y_{cal} - y_{mean})^2} \qquad \text{Equation 1}$$

$$SEC = \sqrt{\frac{\sum^n (y_{cal} - y_{act})^2}{n_{cal} - 1}} \qquad \text{Equation 2}$$

$$SEP = \sqrt{\frac{\sum (y_{pred} - y_{act} - Bias)^2}{n_{val} - 1}} \qquad \text{Equation 3}$$

Table 1. Calibration and validation for starch concentration in meatballs (0-50% starch) using short-wavelength (700-1100 nm) and long-wavelength NIR (1000-2500 nm)

Wavelength(nm)	Meatball	Treatment	*F*	*R²-cal*	*SEC*	*R²-val*	*SECV*	Bias
700-1100	Beef	Smoothing(13)	5	0.91	4.97	0.89	5.55	0.03
700-1100	Chicken	Smoothing(13)	6	0.89	5.65	0.85	6.63	0.04
700-1100	Pork	Smoothing(13)	5	0.88	5.95	0.84	6.85	0.03
1000-2500	Beef	Smoothing(13)	5	0.98	2.26	0.97	2.64	-0.03
1000-2500	Chicken	Smoothing(13)	8	0.98	2.15	0.97	2.63	-0.01
1000-2500	Pork	Smoothing(13)	8	0.99	1.40	0.98	2.37	0.46

F: number of factors; R^2: the coefficient of multiple determination; *SEC*: standard error of calibration, *SECV*: standard error of cross-validation; Bias: the average of differences between reference value and NIR value.

Where n_{cal} is the number of spectra in calibration set, n_{val} is the number of spectra in the validation set, y_{act} is the actual value, y_{mean} is the mean value and y_{pred} is the predicted chemical value.

R^2 indicates the percentage of total variability explained by the PLS model. The standard error of calibration *(SEC)* is a parameter indicating the quality of the NIR calibration fitting of the model. The standard error of cross-validation *(SECV)* and standard error of prediction *(SEP)* point to the standard deviation of the differences between the predicted and measured validation data. This statistical parameter is considered for the best model.

Results and Discussion

Original NIR spectra

The original spectra (absorbance data without statistical pre-treatments) of meatballs at the short and long-wavelength regions were shown in Figure 1. For the short-wavelength region, the spectra shift upward and downward according to the starch contents (at 0%, 20% and 50%) (Figure 1A). For long-wavelength region (Figure 1B), there was no relationship between starch content and NIR absorption. The spectral shift did not happen due to the starch content in the meatballs but might due to the level of light-scattering of the sample.

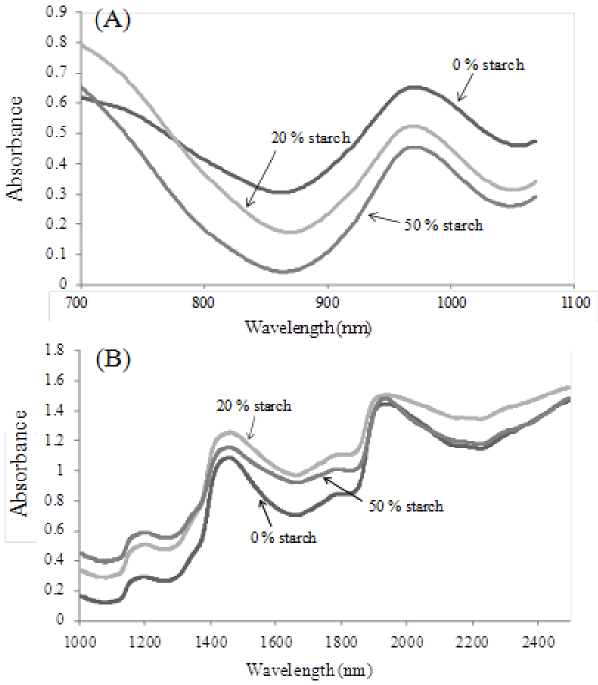

Figure 1. The original spectra of meatballs using short-wavelength NIR (A) and long-wavelength NIR (B)

Data analysis

Short-wavelength NIRS

It was found that the original NIR spectra treated with smoothing technique (13 points) gave the best results. The statistical result of each model was shown in Table 1. For beef meatballs, the model had R^2-*cal* of 0.91, *SEC* of 4.97%, R^2-*val* of 0.89 and *SECV* of 5.55%, respectively. The similar good result was found in other meatball samples. The R^2-*val* was 0.85 with *SECV* of 6.63% and R^2-*val* was 0.84 with *SECV* of 6.85% for chicken and pork meatballs, respectively. As results, it concludes that the short-wavelength NIRS provided a pretty good model to predict the starch contained in meatballs. The regressions coefficient plot of beef meatballs was shown in Figure 2A. It was found that the high regression coefficients were observed in wavelengths of 750 nm and 920 nm that might be linked to the third and second overtone vibration of O-H in water, while the wavelength of 990 nm could be linked to O-H second overtone vibrations of starch (Osborne *et al*., 1993).

Long-wavelength NIRS

The original spectra of each meatball were treated with smoothing or 2nd derivative treatments. To create a PLS model, the treated NIR spectra and the known percentage of starch in the formula (0%, 10%, 20%, 30%, 40% and 50%) were imported by Unscramble software for model calculation. The optimization result was shown in Table 1. It was found that the best

Table 2. Spectra treatments and wavelength optimization for the universal model (0-50 % starch)

Spectra	Treatment	Wavelength (nm)	*F*	R^2-*cal*	*SEC*	R^2-*val*	*SEP*	Bias
Univ. model	Original	1000-2500	8	0.97	2.73	0.95	3.62	0.18
*Univ. model	Smoothing (13)	1000-2500	5	0.98	2.22	0.97	2.67	0.05
Univ. model	2nd derivative	1000-2500	4	0.97	2.92	0.92	4.24	-0.41
Univ. model	Smoothing (13)	1000-2300	8	0.97	2.72	0.95	3.61	0.14
Univ. model	Smoothing (13)	1000-2100	9	0.98	2.21	0.96	3.16	0.15
Univ. model	Smoothing (13)	1000-1900	8	0.97	2.53	0.96	3.27	0.10
Univ. model	Smoothing (13)	1200-2500	6	0.96	3.35	0.93	4.27	0.03
Univ. model	Smoothing (13)	1400-2500	6	0.92	4.77	0.93	4.50	-0.20
Univ. model	Smoothing (13)	1600-2500	6	0.92	4.73	0.93	4.52	-0.02

*was the optimized wavelength giving the best results: *F*: number of factors; R^2: the coefficient of multiple determination; *SEC*: standard error of calibration, *SEP*: standard error of prediction; Bias: the average of differences between reference value and NIR value.

models were obtained when the original spectra were treated with smoothing (13 points) at a wavelength of 1000-2500 nm. The beef meatball model showed a high R^2-*val* of 0.97 and a *SECV* of 2.64%; the chicken meatball model showed an R^2-*val* of 0.97 and a *SECV* of 2.63%; and the pork meatball model showed an R^2-*val* of 0.98 and a *SECV* of 2.37%, respectively.

The regression coefficients and scatter plots of beef meatballs were shown in Figure 2B. It was found that peaks at the starch bands of 1900 nm, 2100 nm and 2252 nm could be observed in the regression coefficient plots (Osborne *et al.*, 1993). On comparing the statistical results of short and long-wavelength NIRS, the long-wavelength NIRS showed higher accuracy for the prediction of starch content than short-wavelength NIRS did. Considering this, the long-wavelength NIRS was used to predict starch concentration for all meatball sources as a universal model.

Universal model

In this step, the universal model was created for starch prediction of all kind meatballs. All original spectra (beef, chicken and pork spectra) were combined together (a total of 180 NIR spectra) and the known starch percentage in formula was used as a chemical value to calculate the PLS model. Each original spectra of meatball were treated with spectra treatments (smoothing or 2nd derivative treatments) and wavelength ranges was optimized. The optimization results were shown in Table 2. The results showed that 1000-2500 nm with smoothing at 13 points had the best statistical results. The best universal model had a high R^2-*cal* of 0.98, a *SEC* of 2.22%, an R^2-*val* of 0.97, a *SEP* of 2.67 % and a Bias of 0.05% with five factors for calculation. Considering this result, it suggested that the universal model could be used to predict starch concentration in all kind meatballs. Although, the beef, chicken and pork meatballs had differences in color, odor and physical properties such as texture, springiness

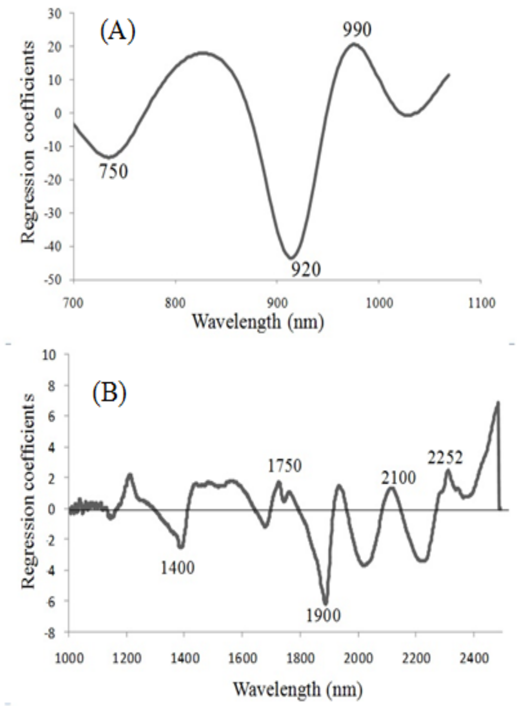

Figure 2. The regression coefficients plot using short-wavelength NIR (A) and the regression coefficients plot using long-wavelength NIR (B) of beef meatball model

and viscosity (data not shown), a good model could be created. This indicated that the differences in physical properties in the meatballs did not affect the determination of starch content by NIRS.

The regression coefficients plot of the universal model for starch concentration in the meatballs was shown in Figure 3A. The high correlation at similar wavelengths was observed as found in the beef meatballs (Figure 2B). In the regression plots, the peaks at the starch bands of 1900 nm, 2100 nm and 2252 nm could be observed as well as the peak at the protein band of 2180nm (Osborne *et al.*, 1993). The scatter plot was shown in Figure 3B. The universal model was created by a separated set (calibration set and validation set). It was found SEP of universal

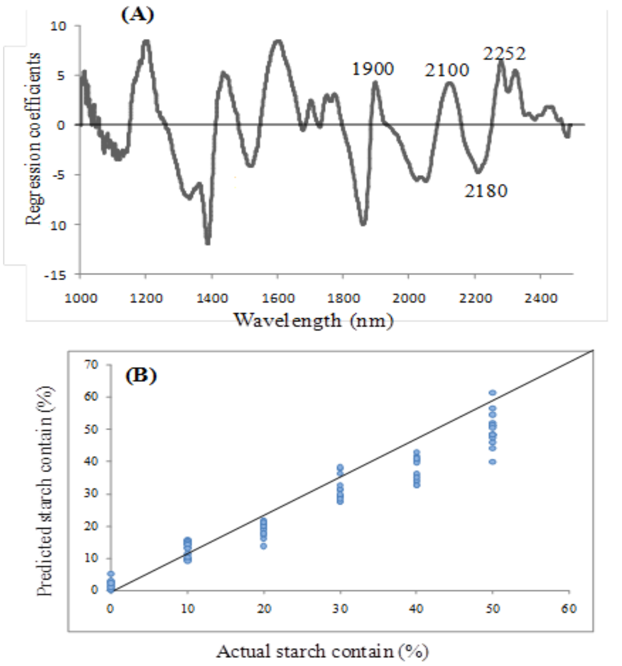

Figure 3. The regression coefficients plot (A) and scatter plot of actual vs. computed starch content of universal model with long-wavelength NIR (B)

model was low (2.67%) as well as SECV of beef (2.64%), chicken (2.63%) and pork (2.37%).

Starch is major component in grain and cereals such as rice, maize and beans. It is a complex polysaccharide consisting of a linear structure or helical chains bonded with polymers of α-(1-4)-linked-D-glucopyranosyl units called amylose and a branch of α-(1-4)-linked-D-glucosyl chain with α-(1-6)-glucosidic linkage called amylopectin. Rationing of amylose and amylopectin is an important factor in the physical properties of starch and the objective to use starch in food production (Hizukuri, 1996). The high correlation wavelength around 1700-1800 nm in this study might be linked to vibration of amylose as described by Fertig *et al.* (2004). Due to the domain structure of starch, it can be detected by NIRS.

In this study, starch content was set in the form of percentage per fresh meats that matched to commercial production of meatballs. The long-wavelength NIRS had a higher accuracy prediction of starch content than short-wavelength NIRS did. Moreover, the universal model of starch can be created and used for good prediction of all kind of meatballs. This finding could be applied for classification of meatball quality in terms of starch content in the formula.

Conclusions

NIRS was used to predict starch content in the formula for meatballs. Several meatballs (beef, chicken and pork meatballs) were used to study at short-wavelength and long-wavelength NIRS. It was found that both short-and long-wavelength NIRS could predict starch contained in meatballs. The differences in physical properties from meat material sources did not affect the detection of starch contained in the meatballs. The results demonstrated that NIRS can predict starch content in the formula of meatballs with high accuracy. The finding from this study will be beneficial for meatball industries in terms of quality control and product classification.

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