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Evaluation of Freshness of Ground Pork Tenderloin by Visible/Near Infrared Spectroscopy

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ABSTRACT

Visible/near-infrared transmittance spectra (600-1368 nm) of the aqueous extracts of 120 ground pork tenderloin samples from Taiwan black-hair hogs were used to correlate with the freshness-relating data including pH, volatile basic nitrogen and aerobic plate count during 7°C storage for 5 days. The spectra in 600-840 nm regions varied during storage with the data highly correlated with the freshness parameters. Four characteristic wavelengths (608, 624, 656 and 732 nm) were selected for freshness evaluation based on partial least square regression (PLSR) model using different freshness criteria for dummy regression. The accuracies of freshness evaluation can reach 90% with the PLSR models excluding the data in Day 3.

Key words: aerobic plate count, chemometrics, freshness, pH, PLSR, pork tenderloin, volatile basic nitrogen

INTRODUCTION

A reliable and rapid method for estimating the freshness of meat is important in disease prevention and consumer protection. Among several freshness indicators, pH, VBN (volatile basic nitrogen) and APC (aerobic plate count) are the most common parameters[1,2]. The pH values reveal the accumulation of lactic acid and other metabolites during glycogen degradation, and are prone to be affected by breed, growth rate, slaughtering conditions, storage, temperature and bacterial contamination[3-6]. VBN values reflect protein decomposition[7,8], and APC values indicate the growth of bacteria[9,10]. However, the measurements of these parameters especially VBN and APC are tedious, time-consuming and often require eco-unfriendly reagents such as perchloric acid and trichloroacetic acid[11,12].

With increased precision and computational skills, modern spectroscopy offered the possibility to obtain more fruitful information from the spectra when combined with some chemometric methods[13-15]. Spectra especially those in the infrared or near infrared regions[16,17] have become more valuable to classify or to retrieve the important chemical information of agricultural products including meat[18-20]. A reflectance spectrum may be very convenient and useful for rapidly obtaining information from a meat sample surface[21-23]. However, for discriminating an unobvious chemical change such as loss of freshness, our preliminary study found that the inhomogeneity and the surface conditions of a meat sample perturbed the precision of the spectral data, which can not be recovered even when we used ground samples or a rotating cup to eliminate the inhomogeneity.

In this study, the aqueous extracts of homogenized ground pork tenderloins from 120 Taiwan black-hair hogs were tested, and the spectral data were used to correlate with the freshness indexes (pH, VBN and APC) and then to evaluate the freshness.

MATERIALS AND METHODS

I. *Preparation of Ground Pork Tenderloin*

Tenderloins of Taiwan black-hair hogs (61 males and 59 females with average body weight of 116.3 ± 9.2 kg) were purchased from a packing plant certified by COA (Council of Agriculture, Taiwan). Chilled (4-5°C) tenderloin samples weighed around 500 g were delivered to the lab within 30 h after slaughtering. Each tenderloin sample was ground four times[24] with a commercial meat grinder (Habart Kitchen Aid Model FPP-A, Ohio, USA) and stored separately in a sterilized

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stomacher bag. To prevent cross-contamination, the grinder was cleaned and disinfected with 75% alcohol every time after homogenizing a sample. The stomacher bags were then stored in a humidity-controlled storage chamber which was set at 7°C to simulate the highest possible storage temperature in local retail markets under subtropical climate. Appropriate amount of ground meat was sampled each day for the following analysis. In this study, day zero was defined as 36 h after slaughtering.

II. *Measurements of pH, VBN and APC*

Five grams of the above ground tenderloin were mixed with 15 mL of distilled water and homogenized at 5,000 rpm for ten seconds in a CR-52F homogenizer (Toyo Keisokuki Co. Osaka, Japan). The homogenate was then filtered through No.1 ashless filter paper (Whatman™, New Jersey, USA), and the clear filtrate was used for VIS/NIR transmittance spectroscopy and pH measurement (Suntex TS-1, Taiwan).

For VBN analyses, 2 g of ground tenderloin were mixed with 18 mL of 2.2% trichloroacetic acid (TCA), stirred for 10 seconds and then allowed to stand for 10 minutes. The upper layer of clear aqueous extract was filtered through No. 1 filter paper and the filtrate was used for analyzing VBN content using a modified Conway's micro-diffusion method[11].

For APC determination, the modified procedures reported by Maturin and Peeler[12,25] were used. Ten grams of the ground pork were mixed with 90 mL of sterilized peptone water in a stomacher bag. It was then agitated for 90 seconds using a stomacher (BagMixer® 400, Intersceience, Nom La Breteche, France). The extract was diluted to different extents and 1 mL of aliquot was transferred into a petri dish containing 15 mL of molten tryptic soy agar (TSA, DIFCO, USA) at 45°C. After solidification, the plates were inverted and incubated at 35°C for 48 h. Colony counts were conducted and the results were expressed as CFU/g (colony forming units per gram meat), or log CFU/g (the logarithm of CFU/g).

III. *VIS/NIR Spectra Acquisition and Chemometric Analysis*

Visible/near-infrared transmittance spectra (600 to 1368 nm at 4-nm interval) were obtained with a dual-beam spectrophotometer, InfraAlyzer 500 (Bran-Lubbe Co. Hamburg, Germany) using distilled water as the reference. Chemometric analysis was based on the absorbance data using principle component analysis (PCA) and partial least square regression (PLSR) conducted under the software, Unscrambler® (version 7.6, CAMO A/S, Trondheim, Norway).

RESULTS AND DISCUSSION

I. *Changes of the pH, VBN, APC Values and Spectra during Storage*

The pH, VBN, APC values of 120 ground pork

Table 1. Changes in three freshness indicators of 120 ground pork tenderloins samples and their linear regression equations during storage at 7°C

Freshness indicator		Storage time, on days						Regression equation[d]
		0	1	2	3	4	5	
pH value	Mean	6.13	6.18	6.19	6.25	6.34	6.44	y = 0.06x + 6.11 R = 0.965
	SD[a]	0.27	0.27	0.27	0.25	0.26	0.25	
	Max[b]	6.78	6.81	6.82	6.80	7.00	6.94	
	Min[c]	5.43	5.55	5.54	5.49	5.48	5.66	
VBN (mg/100g)	Mean	10.68	11.12	12.13	15.37	20.40	26.28	$y = 9.75e^{0.16x}$ R = 0.980
	SD[a]	0.75	1.00	2.09	4.81	7.21	8.97	
	Max[b]	12.60	14.00	22.40	35.00	43.40	53.20	
	Min[c]	9.80	9.80	9.80	11.20	11.20	14.00	
APC (logCFU/g)	Mean	4.60	5.71	7.22	8.42	9.12	9.38	y = 1.01x + 4.87 R = 0.979
	SD[a]	0.78	0.88	0.80	0.61	0.38	0.27	
	Max[b]	6.20	7.60	8.70	9.60	10.20	10.10	
	Min[c]	3.00	3.00	4.50	6.00	7.70	8.30	

[a] Standard deviation.
[b] Maximum.
[c] Minimum.
[d] "x" in regression equation stands for storage day; "y" is the prediction value for the corresponding freshness indicator; "R" is the correlation coefficient.

tenderloin samples were measured during the five-day storage period (Table 1). Due to the inherent buffer capacity of tissue extract and the dilution effect, the mean pH values (6.13-6.44) of the aqueous pork extracts were generally slightly higher than the meat surface pH (5.40-6.00, data not shown). The pH values of the aqueous pork extracts were used for chemometric analysis since the standard deviations are smaller than 0.27.

Compared with pH, the average VBN and APC values obviously increased during the storage. The APC values increased linearly from Day 1 to Day 3, which imply the logarithmic growth of bacteria during this period. The VBN values dramatically rose from Day 2; pork with VBN higher than 15 mg/100g (Day 3) was suggested not to be distributed or sold in Taiwan markets[26]. VBN did reflect the change in biochemical conditions during the storage. From the mentioned regulation and the regression equation shown in Table 1, the predicted allowable storage time for pork tenderloin is 2.62 days at 7°C. On Day 5, almost all samples smelled rotten. Again, from CAS[12,26] for APC (< 7.5 log CFU/g) and the regression equation in Table 1, the predicted allowable storage time is 2.58 days at 7°C. The above two parameters suggest that the meat starts changing (spoiling) around Day 3, so do the absorption spectra (Figure 1).

As also shown in the spectra (Figure 1), no obvious changes were observed in the first two days (Day 0 to Day 2). From Day 3, the absorbance data were generally lower than those obtained in the fresh states especially in the wavelength regions lower than 840 nm or higher than 1100 nm. Absorption at wavelength shorter than 600 nm was not considered since particles existed in the filtrate tend to increase the absorbance. As the spectral data of Day 4 and Day 5, although a characteristic absorption peak was not found, it is still possible to discriminate the "fresh" spectra from the "spoiled" ones if a trustful statistical result was obtained from sufficient numbers of sample.

Table 2. The results of PC analyses and PLSR models between the absorbance spectra and their corresponding data for freshness indicators of ground pork tenderloin during storage at 7°C

Wavelength range (nm)		Calibration (n = 480)				Validation (n = 240)		
		pH	VBN	APC		pH	VBN	APC
600-1368	R[a]	0.92	0.88	0.62	R[a]	0.78	0.84	0.61
	SEC[b]	0.13	3.78	2.01	SEP[c]	0.20	5.26	1.75
	PCs[d]	7	15	9	PCs[d]	7	15	9
600-1368 The 1st difference	R[a]	0.92	0.86	0.50	R[a]	-	-	-
	SEC[b]	0.14	4.10	11.46	SEP[c]	-	-	-
	PCs[d]	7	15	9	PCs[d]	-	-	-
600-1368 the 2nd difference	R[a]	0.87	0.84	0.55	R[a]	-	-	-
	SEC[b]	0.17	4.40	2.14	SEP[c]	-	-	-
	PCs[d]	7	15	9	PCs[d]	-	-	-
600-840	R[a]	0.90	0.86	0.81	R[a]	0.71	0.85	0.68
	SEC[b]	0.14	3.69	1.37	SEP[c]	0.21	5.54	1.82
	PCs[d]	5	7	20	PCs[d]	5	7	20
1100-1368	R[a]	0.85	0.58	0.61	R[a]	0.76	0.32	0.41
	SEC[b]	0.16	5.48	1.69	SEP[c]	0.21	9.05	1.86
	PCs[d]	7	9	11	PCs[d]	7	9	11
600-840 plus 1100-1368	R[a]	0.91	0.87	0.80	R[a]	0.76	0.86	0.64
	SEC[b]	0.13	3.60	1.40	SEP[c]	0.20	5.19	1.71
	PCs[d]	6	8	15	PCs[d]	6	8	15

[a] Correlation coefficient.
[b] Standard error of calibration.
[c] Standard error of validation.
[d] Numbers of principal component.
- Data are not analyzed.

II. *Chemometric Analyses of the VIS/NIR Spectra*

For 120 samples each with 6 different storage periods (Day 0 to Day 5), totally 720 spectra were analyzed. Similar to the approach adopted by Irudayara and Sivakesava[27], randomly two-thirds of the spectra data (n = 480) and their corresponding freshness indicators were used for calibration, and the remaining one-third (n = 240) were used for validation. A leave-one-out, full-cross validation PLSR method was used to prevent over-fitting. These results are shown in Table 2.

In the upper part of Table 2, full spectra (600-1368 nm) with their first and second difference spectra were correlated with three freshness parameters (pH, VBN and APC). The correlation coefficients for APC are significantly lower than those for VBN since APC is expressed as the logarithmic values of bacterial count that tend to become saturated. The differential spectra did not show benefits in the correlation. Considerable improvements were obtained by correlating the data in different wavelength regions (the lower part of Table 2); the data in the wavelength range between 840 and 1100 nm were excluded in the statistic treatments since the segment of spectrum did not significantly vary during the storage (Figure 1). Satisfactory results ($R > 0.8$)[28] were obtained in the wavelength region of 600-840 nm. For a quicker chemometric analysis, four characteristic wavelengths (608, 624, 656 and 732 nm) in the above wavelength region were selected by the first and second difference methods of Savitzky-Golay and the resulting correlation coefficient for validation was 0.83 (Figure 2).

III. *Freshness Evaluation*

The aforementioned four characteristic wavelengths (608, 624, 656 and 732 nm) were selected for PLSR model development. To eliminate ambiguity, the data on Day 3 were excluded since the samples started spoiling on Day 3; the remaining 600 spectra and the corresponding data sets were used for the model development.

To adapt dummy regression[29,30], data from day 0 to day 2 were pooled and assigned as dummy number "1" for "fresh", and those from day 4 and 5 were assigned as "2" (for spoiled). With 80 data sets selected randomly from each day (out of 120 data sets/day) of day 0 to day 2, totally 240 data sets were used in the "fresh" training. Similarly 160 data sets from day 4 and 5 were used for establishing the "spoiled" training. The remaining 120 (day 0 to day 2) and 80 (day 4 to day 5) data sets were used for validation. The results of 200 validation processes are summarized in Figure 3; pork samples with a dummy value less than 1.5 would be considered "fresh", whereas a value greater than or equal to 1.5 would be considered "spoiled". The accuracy for prediction was 90.5% (Table 3, Model E). Other optional prediction results based on different criteria are also shown in the other models (Model A, B, C and D) in Table 3.

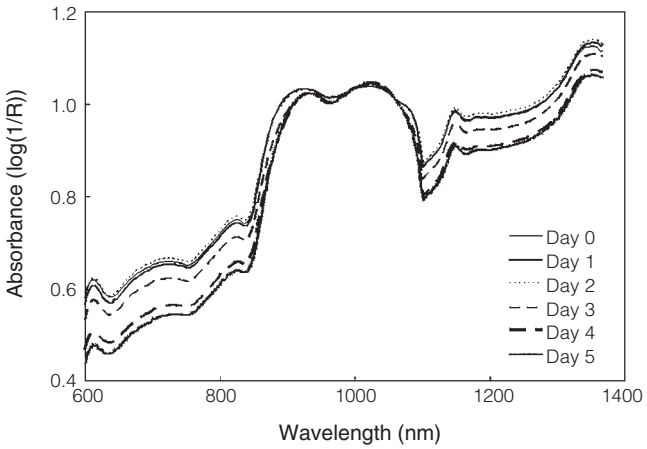

Figure 1. Changes in visible and near-infrared transmittance spectra of the aqueous extracts of ground pork tenderloins during storage at 7°C. Each absorbance spectrum is the average of the spectral data obtained from 120 tenderloin samples.

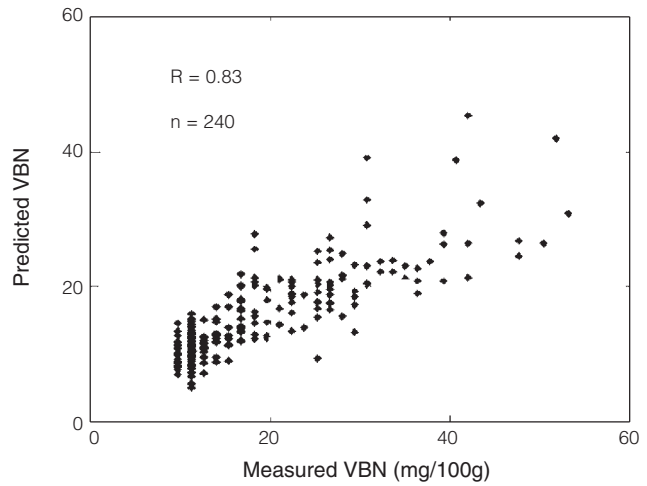

Figure 2. Validation results of 240 absorbance data sets at four characteristic wavelengths (608, 624, 656 and 732 nm) showing the correlation between the measured and predicted VBN values.

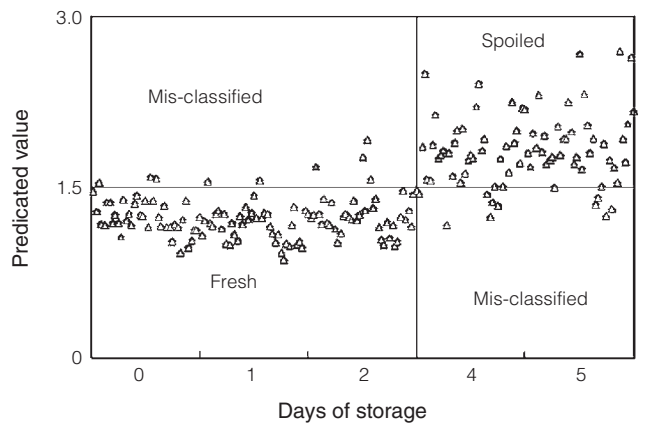

Figure 3. Freshness evaluation of 200 ground pork tenderloin samples using PLSR model E (Table 3).

Table 3. Summary of criteria and sample sizes for five PLSR calibration and validation models and their resultant accuracy of freshness evaluation for ground pork tenderloin during storage at 7°C

PLSR model and criteria (in parentheses)	Sample numbers				Accuracy (%)[#]
	Total	Calibration	Validation	Misclassified	
A: VBN (15 mg/100g)					
Fresh	444	298	146	8	94.5
Spoiled	276	182	94	28	70.2
Total	720	480	240	36	85.0
B: APC (7.5 logs CFU/g)					
Fresh	313	167	146	19	87.0
Spoiled	407	313	94	31	67.0
Total	720	480	240	50	79.2
C: VBN and APC					
Fresh	313	209	104	10	90.4
Spoiled	273	182	91	23	74.7
Total	586	391	195	33	83.1
D: Excluding Day3's data					
Fresh (VBN < 15 and ≦ d2)	307	205	102	4	96.1
Spoiled (VBN ≧ 15 and≧ d4)	212	141	71	18	74.6
Total	519	346	173	22	87.3
E: Excluding Day3's data					
Fresh (≦ d2)	360	240	120	9	92.5
Spoiled (≧ d4)	240	160	80	10	87.5
Total	600	400	200	19	90.5

[#] Percentage of accuracy = 100 × (validation numbers - misclassified numbers)/(validation numbers).

CONCLUSIONS

Resolved by improved instrumental precision and computational skills, conventional spectroscopy such as UV or NIR has become more informative and practical even when deals with unpurified biomedical samples. To discriminate an insignificant chemical change in sample matrix such as loss in freshness, a simple pretreatment such as extraction with an eco-friendly solvent will be helpful in reducing the problems of sample inhomogeneity during the acquisition of spectral data. The present study revealed the possibility of using the above approach in diverse and complicated biomedical applications, and the data showed strong correlations with the important freshness parameters of meat.

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REFERENCES

1. Chen, C. M., Lin, L. C. and Yen, G. C. 1994. Relationship between changes in biogenic amine contents and freshness of pork during storage at different temperatures. J. Chin. Agri. Chem. Soc. 32: 47-60.
2. Shaw, J. T., Lou, C. Y., Chen, L. C., Liu, C. C., Ang, S. L. and Su, S. J. 2004. Studying the relationship between pork freshness indices and near-infrared spectra. J. Agri. Machin. 13: 27-36. http://www.bime.ntu.edu.tw/ciam/
3. Devine, C. E., Graafhuis, A. E., Muir, P. D. and Chrystall, B. B. 1993. The effect of growth rate and ultimate pH on meat quality of lambs. Meat Sci. 35: 63-77.
4. Brown, S. N., Warriss, P. D., Nute, G. R., Edwards, J. E. and Knowles, T. G. 1998. Meat quality in pigs

subjected to minimal preslaughter stress. Meat Sci. 49: 257-265.
5. Hambrecht, E., Eissen, J. J. and Verstegen, M. W. A. 2003. Effect of processing plant on pork quality. Meat Sci. 64: 125-131.
6. Gregory, N. G. 2005. Recent concerns about stunning and slaughter. Meat Sci. 70: 481-491.
7. Vinci, G.. and Antonelli, M. L. 2002. Biogenic amines: quality index of freshness in red and white meat. Food Control 13: 519-524.
8. Fanco, I., Prieto, B., Cruz, J. M., Lopez, M. and Carballo, J. 2002. Study of the biochemical changes during the processing of Androlla, a Spanish dry-cured pork sausage. Food Chem. 78: 339-345.
9. Jay, J. M. 1992. Indicators of food microbial quality and safety. In "Modern food microbiology". pp. 413-433. Chapman and Hall, New York, U.S.A.
10. Al-Bachir, M. and Mehio, A. 2001. Irradiated luncheon meat: microbiological chemical and sensory characteristics during storage. Food Chem. 75: 169-175.
11. Chinese National Standard (CNS). 1995. Method of volatile basic nitrogen determination for frozen fishes. Manual of Chinese National Standard, Code 1451-N6029. Bureau of Central Standard, Taiwan, R.O.C. http://www.cnsonline.com.tw/en/
12. Chinese National Standard (CNS). 1991. Method of test for food microbiology determination of bacteria. Manual of Chinese National Standard, Code 10890-N6186. Bureau of Central Standard, Taiwan, R.O.C. http://www.cnsonline.com.tw/en/
13. Williams, P. and Norris, K. 2001. Near-infrared technology in the agricultural and food industries. 2nd ed. American Association of Cereal Chemists Inc. Minnesota, U.S.A.
14. Chan, D. E., Walker, P. N. and Mills, E. W. 2002. Prediction of pork quality characteristics using visible and near-infrared spectroscopy. Trans. ASAE 45: 1519-1527.
15. Becker, E. M., Christensen, J., Frederiksen, C. S. and Haugaard, V. K. 2003. Front-face fluorescence spectroscopy and chemometrics in analysis of yogurt: rapid analysis of riboflavin. J. Dairy Sci. 86: 2508-2515.
16. Ellis, D. I., Broadhurst, D., Kell, D. B., Rowland, J. J. and Goodacre, R. 2002. Rapid and quantitative detection of the microbial spoilage of meat by Fourier transform infrared spectroscopy and machine learning. Appl. Environ. Microbiol. 68: 2822-2828.
17. Lin, M., Al-Holy, M., Mousavi-Hesary, M., Al-Qadiri, H., Cavinato, A. G. and Rasco, B. A. 2004. Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600-1100 nm). Lett. Appl. Microbiol. 39: 148-155.
18. Xing, J., Ngadi, M., Gunenc, A., Prasher, S. and Gariepy, C. 2007. Use of visible spectroscopy for quality classification of intact pork meat. J. Food Eng. 82:135-141
19. Ortiz-Somovilla, V., España-España, F., Gaitán-Jurado, A. J., Pérez-Aparicio, J. and De Pedro-Sanz, E. J. 2007. Proximate analysis of homogenized and minced mass of pork sausages by NIRS. Food Chem. 101: 1031-1040.
20. Osborne, B. G., Fearn, T. and Hindle, P. H. 1993. Practical Spectroscopy with Application in Food and Beverage Analysis. Longman Scientific and Technical. Harlow, U.K.
21. Prieto, N., Andrés, S., Giráldez, F. J., Mantecón, A. R. and Lavín, P. 2008. Ability of near infrared reflectance spectroscopy (NIRS) to estimate physical parameters of adult steers (oxen) and young cattle meat samples. Meat Sci. 79: 692-699.
22. Geesink, G. H., Schreutelkamp, F. H., Frankhuizen, R., Vedder, H. W., Faber, N. M., Kranen, R. W. and Gerritzen, M. A. 2003. Prediction of pork quality attributes from near infrared reflectance spectra. Meat Sci. 65: 661-668.
23. Hoving-Bolink, A. H., Vedder, H. W., Merks, J. W. M., de Klein, W. J. H., Reimert, H. G. M., Frankhuizen, R., van den Broek,W. H. A. M. and en Lambooij, E. 2005. Perspective of NIRS measurements early post mortem for prediction of pork quality. Meat Sci. 69: 417-423.
24. Beebe, R. M., Lay, E. and Eisenberg, S. 1989. Homogeneity of meats prepared for analysis with a commercial food processor: collaborative study. J. Assoc. Off. Anal. Chem. 12: 777-783.
25. Maturin, L. J. and Peeler J. T. 2001. Chapter 3: Aerobic plate count. In "Bacteriological Analytical Manual". U.S. Food and Drug Administration. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063346.htm
26. Chinese Agriculture Standard (CAS). 1998. Manual-Meat Class. Foundation of Taiwan Meat Development, Taiwan, R.O.C. http://www.cas.org.tw/en/
27. Irudayara, J. and Sivakesava, S. 2001. Detection of adulteration in honey by discriminant analysis using FITR spectroscopy. Trans. ASAE 44: 643-650.
28. Hruschka, W. R. 2001. Data analysis: wavelength selection methods. In "Near-Infrared Technology in the Agricultural and Food Industries". 2nd ed. pp.39-58. Williams P and Norris K. ed. American Association of Cereal Chemists Inc. Minnesota, U.S.A.
29. Ding, H. B., Xu, R. J. and Chan, D. K. 1999. Identification of broiler chicken meat using a visible/near-infrared spectroscopic technique. J. Sci. Food Agric. 79: 1382-1388.
30. Downey, G. and Beauchene, D. 1997. Discrimination between fresh and frozen-then-thawed beef m. longissimus dorsi by combined visible-near infrared reflectance spectroscopy: A feasibility study. Meat Sci. 45: 353-363.