Indian Journal of Animal Sciences **89**(7): 786–790, July 2019/Article
https://doi.org/10.56093/ijans.v89i7.92052

Fluorescence spectroscopy for accurate and rapid prediction of meat composition

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Received: 15 June 2018; Accepted: 4 December 2018

ABSTRACT

The potential of fluorescence spectroscopy was assessed to study cow, goat, sheep and yak meat. Meat samples were taken from muscles, viz. *Gluteus medius* (GM), *Longissimus dorsi* (LD) and *Semitendinosus* (ST). The moisture, fat and protein content of meat samples were measured. The emission fluorescence spectra of tryptophan (305–500 nm), riboflavin (410–700 nm) and vitamin A (340–540) were recorded directly on meat samples at 290, 382 and 322 nm, respectively. Principal component analysis (PCA), partial least squares regression (PLSR) and partial least squares discriminant analysis (PLSDA) were applied to process the spectra obtained. Moisture content with R^2=0.94, protein content with R^2=0.86, and fat content with R^2=0.91 were predicted from the fluorescence emission spectra. The PLSDA applied at 410–700 nm fluorescence spectra showed 100, 100, 94.4 and 92.6% of discrimination for cow, goat, sheep and yak meat, respectively. This study demonstrates that fluorescence spectroscopy has a potential for the accurate, non-destructive and rapid prediction of meat composition and it could replace existing traditional analytical methods.

Key words: Animal species, Chemical parameters, Chemometrics, Fluorescence spectroscopy, Meat

Meat plays an important role in supplying excellent nutrients. The elucidation of the health-promotion role of meat requires databases of values and analytical techniques. Many analytical techniques are available to evaluate meat nutrients and create nutrients databases. Nevertheless, these techniques are time-consuming, destructive, laborious, and require highly skilled operators and are not easily adapted to on-line monitoring (Sahar and Dufour 2015, Abasi *et al.* 2018). There is a continuously growing demand for high-speed, non-destructive, simple-in-use and accurate techniques able to replace visual and manual inspections yet practiced in meat industry at present (Abasi *et al*. 2018).

Fluorescent spectroscopy based techniques have become increasingly important in the study of meat and meat products (Kulmyrzaev *et al.* 2007). Fluorescence spectroscopy is considered as a tool to classify bovine muscles according to their chemical and rheological characteristics (Sahar *et al*. 2009), for determination of composites of meat (Wold *et al.* 1999, Skjervold *et al.* 2003, Sahar and Dufour 2015), to study tenderness of meat (Egelandsdal *et al*. 2002, Sahar and Dufour 2015) and to determine quinolone antibiotics in meat (Pagani and Ibanez 2018).

The quality of meat products depends on the composition of the meat. In order to produce meat products of high quality, it is important to maintain strict control in all stages of manufacturing processes. The on-line monitoring and determination of fat, moisture and protein contents in industrial scale will be helpful in rapid screening of large number of samples, quick product recall and produce meat products with high quality. The objective of this study was to assess the potential of fluorescence spectroscopy to predict moisture, protein and fat contents of cow, yak, goat and sheep meat. The potential of fluorescence spectra to discriminate different meat samples in relation with biological factors (muscle type and animal type) was also examined.

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MATERIALS AND METHODS

Meat samples: The experimental muscles were supplied by a local farm (Bishkek, Kyrgyz Republic). Approximately 2–2.5 year-old female cows, yaks, goats and sheep were selected to consider the animal type factor. *Gluteus medius* (GM), *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles were removed from carcasses after slaughter. Each muscle was cut into 2 pieces to conduct 2 kinds of experiments, chemical composition measurements and fluorescence spectral measurements. The muscle samples were identified, vacuum-packaged in polyethylene bags and stored for 3 days at 4°C and were kept at –20°C until measurements. The samples were thawed at 4°C before analysis.

Chemical analysis: The moisture, fat and protein of meat samples were measured (AOAC 2000). Analysis was performed in triplicate for each sample and the average values were taken.

Fluorescence spectroscopy: Fluorescence spectra were recorded using a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon, USA) provided with a single position (56°). The meat samples were cut into rectangle with dimensions of 2×3 cm. The meat specimens were placed between quartz plates and transferred into the cell holder of the spectrofluorometer. The emission spectra of tryptophan in the range of 305–500 nm, riboflavin in the range of 410–700 nm and vitamin A in the range of 340–540 nm were recorded with the excitation at 290 nm, 382 nm and 322 nm, respectively. Each spectrum was recorded in 6 copies on different samples. Total 216 spectra (3 types of spectra, 3 types of muscles, 4 animal types, and 6 repetitions) were recorded.

Multivariate statistical analysis: The statistical processing were used to derive relevant information from the fluorescence spectral data allowing prediction of chemical composition and discrimination parameters. In order to reduce scattering effects, the fluorescence spectra were normalized by reducing the area under each spectrum to a value of 1 according to Bertrand and Scotter (1992). Normalization of the fluorescence spectra was conducted using a custom-designed algorithm written in MatLab (The MathWorks Inc., MA, USA).

Chemical data tables were standardized to assure zero mean and unit variance of each column. The experimental data were arranged in 4 data tables, viz. emission spectra of tryptophan, emission spectra of vitamin A, emission spectra of riboflavin, and chemical composition.

Principal component analysis (PCA) was applied to the normalized fluorescence spectral data to obtain a map describing chemical variations between the samples studied. Partial least squares regression (PLSR) was applied to predict the chemical composition of the meat samples from fluorescence data. PLSR searches the relationship and interdependence of two or more random variables and describes their common structure. The accuracy of the regression is expressed with a correlation coefficient (R^2). Partial least square discriminant analysis (PLSDA) is one of the classification methods and rely on the PLS model. PLSDA was applied to predict membership of an individual to a group defined as a preliminary (Westerhuis *et al.* 2008).

The custom-designed versions of PCA, PLSR and PLSDA ('SAISIR' package) programmed in MatLab (The MathWorks Inc., MA, USA) were utilized in the statistical data treatment.

RESULTS AND DISCUSSION

Chemical composition: The data show a wide range of variation in the chemical content of the samples (Table 1). Yak meat samples had the greatest moisture and the lowest fat. Sheep meat showed the greatest fat, and the lowest protein content. The high-fat content was found in sheep meat, followed by the samples of cow meat. The less meat samples contained fat, the more moisture was found in it. Similar results were reported by Sahar and Dufour (2015). Goat meat exhibited the greatest protein content (21.24–22.71%) and relatively low fat (4.92–8.62%). GM muscles contained the highest percentage of fat among the muscles, whereas LD muscles contained the lowest fat percentages. Contents of protein were higher in ST muscles (Table 1). The protein content was inversely proportional to the fat content of the samples. The values were similar to those reported by Sahar *et al.* (2009) and Sahar and Dufour (2015).

Table 1. Chemical composition of the meat samples

		Moisture (%)	Protein (%)	Fat (%)
Cow	GM[a]	63.68±0.19	19.92±0.34	15.62±0.32
	LD[a]	68.05±0.20	19.87±0.29	10.87±0.12
	ST[a]	65.19±0.29	20.66±0.23	13.01±0.17
Goat	GM	69.85±0.49	21.24±0.38	8.62±0.18
	LD	69.71±0.49	21.98±0.38	6.56±0.27
	ST	70.08±0.49	22.71±0.38	4.92±0.31
Sheep	GM	64.11±0.20	17.88±0.18	16.83±0.29
	LD	66.54±0.32	18.39±0.24	13.47±0.34
	ST	65.29±0.44	19.22±0.14	14.16±0.11
Yak	GM	71.06±0.14	20.55±0.24	7.09±0.25
	LD	73.07±0.13	20.83±0.25	4.85±0.16
	ST	71.32±0.32	22.59±0.24	4.66±0.33

[a]*Gluteus medius* (GM), *Longissimus dorsi* (LD) and *Semiten dinosus* (ST).

Fluorescence properties: Fluorescence spectra were different between meat samples of various animals (Fig. 1 A-C). The emission spectra in the range of 305–500 nm of the LD muscle exhibited a maximum peak in 371, 371, 372 and 372 nm recorded with the cow, yak, sheep and goat meat, respectively (Fig. 1A). These spectra could be originated from tryptophan residues in proteins (Lakowicz 2006). So, the spectrum recorded on the meat sample following excitation at 290 nm may be considered as a characteristic fingerprint, which allows the sample to be identified. The spectra collected in the range of 340–540 nm wavelengths of the LD muscles showed different shapes and intensities among the samples with two maxima observed at 386–394 nm and 461–468 nm (Fig. 1B). Similar peaks were found in fluorescence spectra obtained from beef muscles and were assigned to vitamin A (Skjervold *et al.* 2003, Kulmyrzaev *et al.* 2007, Sahar *et al.* 2009, Sahar and Dufour 2015).

The spectra collected in the range of 410–700 nm had a maximum intensity at about 441–467 nm with a shoulder at 418 nm (Fig. 1C). Additionally, the width and intensities of the fluorescence spectra differed from one meat sample to the other. Wold *et al.* (1999) and Egelandsdal *et al.* (2002) showed that 380 nm is the best excitation wavelength for the quantification of connective tissue. Identification and quantification of different components of muscle connective tissue, collagen and elastin, were investigated by intrinsic fluorescence spectroscopy (Egelandsdal *et al.* 2002). Elastin and collagen type 5 showed the most powerful fluorescence with peaks at 440 and 475 nm after excitation at 380 nm (Skjervold *et al.* 2003). The variation of environment affects the maximum intensity of riboflavin emission (Ladokhin

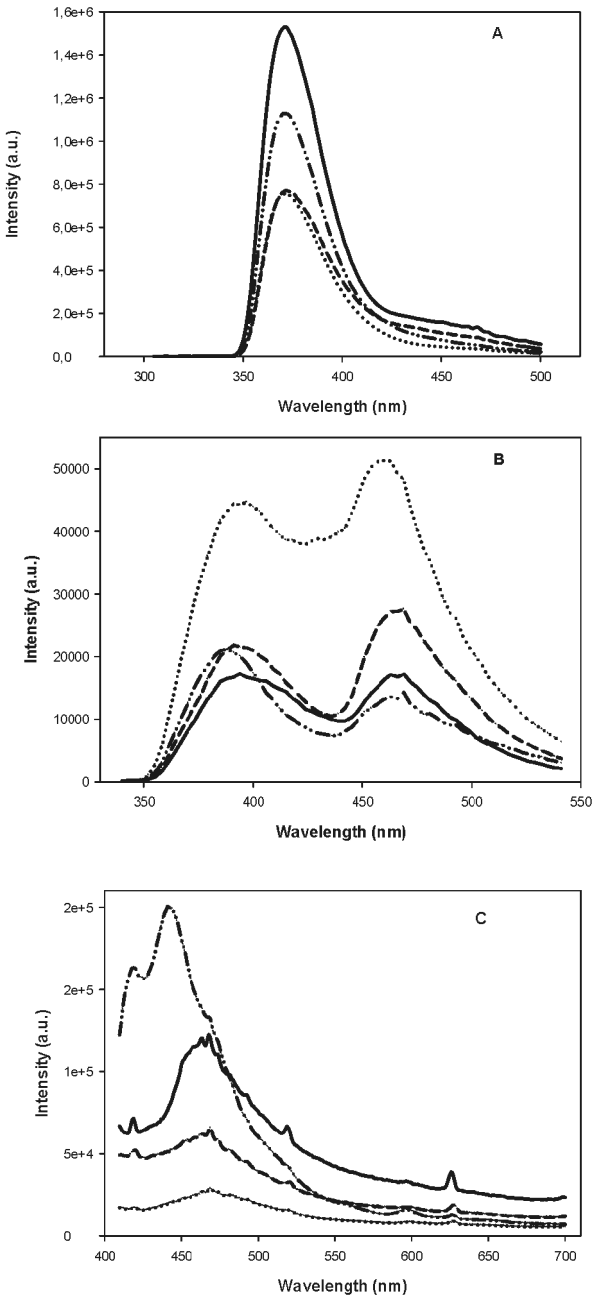

Fig. 1. (A) Tryptophan emission spectra, (B) vitamin A emission spectra, and (C) riboflavin emission spectra of the *Longissimus dorsi* (LD) muscle of the cow (—), goat (••••), sheep (– – –) and yak (–••–••) meat.

2000, Lakowicz 2006). The shapes of spectra distinctly changed depending on the moisture content. As the moisture content increased, maximum intensity of riboflavin emission increased and maximum intensity wavelength shifted from 442 nm at 71.82% of moisture to 467 nm at 65.64% of moisture (Fig. 1C). The increase of riboflavin emission spectra could be explained by the increase of water content of samples (Fig. 1C). Therefore, the riboflavin emission spectra could be used as an indicator group for moisture content. Similar spectral patterns were also found in the riboflavin emission spectra of the GM and ST muscles of cow, goat, sheep and yak meat (data not shown).

Multivariate statistical analysis: PCA was applied to normalized fluorescence spectral data recorded in 305–500 nm, 410–700 nm and 340–540 nm ranges in order to investigate the variations among meat samples, to determine whether the groups showed different spectral characteristics and similarities. The PCA score plot obtained with the riboflavin emission spectra of the meat samples is presented in Fig. 2. Principal component A1 and A2 explained 83.5% and 12% of the total variance of the spectra, respectively. The discrimination pattern can be explained by differences in the chemical composition of the samples (Table 1). The spectra of cow meat generally were scored positively relative to the principal component A1, while the yak meat spectra were scored negatively. Yak meat samples were rather well separated from the other meat samples according to the principal component A1 which accounted for 83.5% of the total variance (Fig. 2). Such a separate location of yak meat samples can be derived from the high moisture content. Yak meat had higher moisture content than other meat samples (Table 1). Disposition of the meat samples relative to A2 indicated that cow, goat and sheep meat samples were almost similar taken into account the corresponding riboflavin emission spectra. It can be seen that different locations of the samples were observed considering to the chemical content, which makes it possible to classify meat samples according to the animal species. Similar results were obtained when PCA was applied to tryptophan and vitamin A spectra (data not shown).

The PLSR algorithm with cross-validation was used to develop regression models of the chemical composition and fluorescence spectra of the meat samples. The numbers of PLSR factors used for the final models were the numbers giving first local minimum for the root mean squared error of validation (RMSEV). The results of PLSR carried out on spectral and chemical data are reported in Table 2. The best regression model including 13 components (R^2= 0.94) to predict moisture content was obtained applying PLSR on riboflavin emission spectra. Tryptophan had the strongest fluorescence quantum yield among three fluorescent amino acids found in proteins, therefore protein fluorescence usually refers to the fluorescence emission of the tryptophan (Ladokhin 2000). Consequently, tryptophan emission spectra showictive value (R^2 = 0.86) for protein content of the samples. The results with this technique were quite similar to the results of Dufour and Frencia (2001).

The prediction of fat was much better from vitamin A emission spectra (R^2=0.91) than from tryptophan and riboflavin emission spectra (0.54 and 0.69, respectively). The 340–540 nm range has been proven to be a good indicator of fat content in food (Lakowicz 2006, Kulmyrzaev *et al.* 2007). The high correlation between the data measured by the traditional and fluorescent spectroscopic methods indicates that the latter is a very sensitive technique to predict meat chemical compositions.

PLSDA was used to assess the ability of the fluorescent spectra to distinguish (as a function of muscle type and

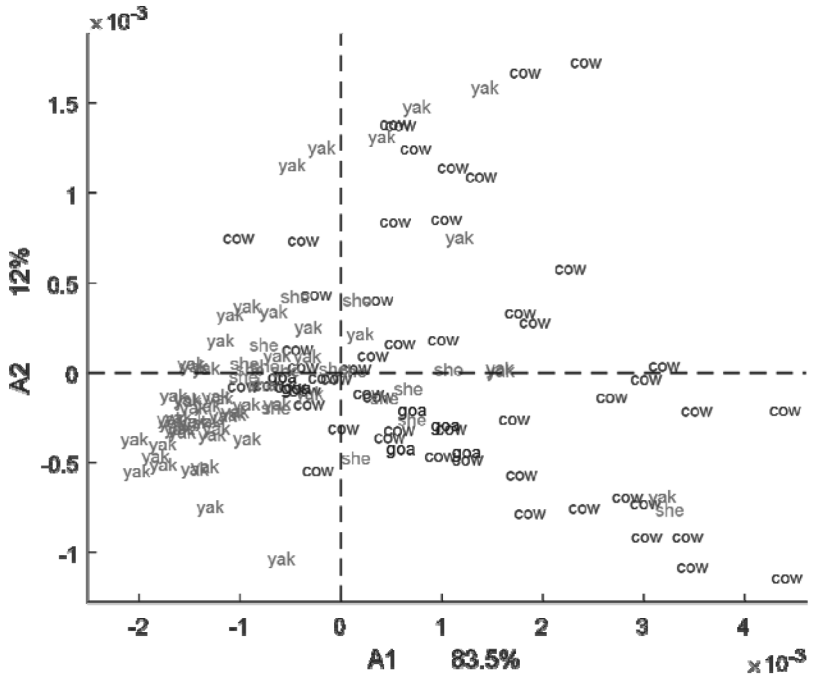

Fig. 2. PCA similarity map determined by principal components A1 (83.5%) and A2 (12%) of riboflavin emission spectra.

Table 2. PLSR statistics to predict chemical contents of the meat samples from fluorescence spectra

Parameter	Tryptophan emission spectra		Vitamin A emission spectra		Riboflavin emission spectra	
	R^2	PLS components	R^2	PLS components	R^2	PLS components
Moisture (%)	0.57	6	0.59	9	0.94	13
Protein (%)	0.86	8	0.62	9	0.65	11
Fat (%)	0.54	8	0.91	11	0.69	14

animal type) meat samples. The results of PLSDA applied to fluorescence spectra are given in Table 3. The emission spectra in the range of 410–700 nm with 100, 100, 94.4 and 92.6% of correctly distinguished samples according to animal type. Moreover, the emission spectra in the range of 305–500 nm showed a good discrimination for meat samples. Out of 54 samples of cow meat,11 were recognized as originated from goat and yak meat, while 2 out of 54 samples of yak meat were recognized as the samples of cow meat resulting in 96.3% of the yak meat samples being discriminated correctly depending on animal type. PLSDA applied on the 340–540 nm emission spectra made it possible to discriminate the meat samples not exceeding 83.3%. Also, PLSDA allowed distinguishing the meat samples depending on muscle type with accuracies not less than 89% (data not shown). The results with this technique can be used to identify animal species of origin of meat samples to eliminate problems related to religious and economic aspects.

Fluorescence spectroscopy coupled with multivariate statistical tools was successfully used to develop PLS regression models to predict moisture, protein and fat content of the cow, goat, sheep and yak meat. In addition, by means of multivariate statistical technique, it was demonstrated that tryptophan, riboflavin, and vitamin A emission spectra allow discriminating meat samples with respect to muscle type and animal type. Therefore, fluorescence spectroscopy as a fast, non-destructive and

Table 3. PLSDA conducted on spectral data of the investigated meat samples.

	Cow	Goat	Sheep	Yak	Percentage of good classification
Emission spectra (410–700 nm)					
Cow	54	–	–	–	100
Goat	–	54	–	–	100
Sheep	–	3	51	–	94.4
Yak	1	–	3	50	92.6
Emission spectra (305–500 nm)					
Cow	43	4	–	7	79.6
Goat	–	45	9	–	83.3
Sheep	–	3	48	3	88.9
Yak	2	–	–	52	96.3
Emission spectra (340–540 nm)					
Cow	37	–	–	17	68.5
Goat	9	36	2	7	66.7
Sheep	–	9	45	–	83.3
Yak	9	–	–	45	83.3

on-line control technique may successfully be applied in the meat industry. In order to test its robustness, the technique developed in this study will be examined using greater number of meat samples.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support rendered by Kyrgyz-Turkish Manas University.

REFERENCES

Abasi S, Minaei S, Jamshidi B and Fathi D. 2018. Dedicated non-destructive devices for food quality measurement: A review. *Trends In Food Science and Technology* **78**: 197–205.

AOAC. 2000. *Meat and Meat Products*. Official Methods of Analysis. pp. 931–49. (Ed) William Horwitz. Association of Official Analytical Chemists, Maryland.

Bertrand D and Scotter C N G. 1992. Application of multivariate analyses to NIR spectra of gelatinized starch. *Applied Spectroscopy* **46**: 1420–25.

Dufour E and Frencia J P. 2001. Les Spectres de Fluorescence Frontale-uneEmpreinteDigitale de la Viande. *Viandes and ProduitsCarnés* **22**: 9–14.

Egelandsdal B, Wold J P, Sponnich A, Neegard S and Hildrum K I. 2002. On attempts to measure the tenderness of *Longissimus dorsi* muscles using fluorescence emission spectra. *Meat Science* **60**: 187–202.

Kulmyrzaev A A, Karoui R, De Baerdemaeker J and Dufour E. 2007. Infrared and fluorescence spectroscopic techniques for the determination of nutritional constituents in foods. *International Journal of Food Properties* **10**: 299–320.

Ladokhin A S. 2000. Fluorescence spectroscopy in peptide and protein analysis. *Encyclopedia of Analytical Chemistry*. pp. 5762–79. (Ed) Meyers R A. John Wiley & Sons Ltd, Chichester.

Lakowicz J R. 2006. *Fluorophores. Principles of fluorescence Spectroscopy*. pp. 63–93. (Ed) Lacowicz JR. Plenum Press, New York.

Pagani A P and Ibanez G A. 2018. Analytical approach for the simultaneous determination of quinolones in edible animal products. Modeling pH-modulated fluorescence excitation-emission matrices four-way arrays. *Talanta* **192**: 52–60.

Sahar A and Dufour É. 2015. Classification and characterization of beef muscles using front-face fluorescence spectroscopy. *Meat Science* **100**: 69–72.

Sahar A, Boubellouta T, Lepetit J and Dufour É. 2009. Front-face fluorescence spectroscopy as a tool to classify seven bovine muscles, according to their chemical and rheological characteristics. *Meat Science* **83**: 672–77.

Skjervold P O, Taylor R G, Wold J P, Berge P, Abouelkaram S, Culioli J and Dufour E. 2003. Development of intrinsic fluorescent multispectral imagery specific for fat, connective tissue, and myofibers in meat. *Journal of Food Science* **68**(4): 1161–69.

Westerhuis J A, Hoefsloot H C J, Smit S, Vis Daniel J, Smilde A K, van Velzen E J J, van Duijnhoven J P M and van Dorsten F A. 2008. Assessment of PLSDA cross validation. *Metabolomics* **4**: 81–89.

Wold J P, Lundby F and Egelandsdal B. 1999. Quantification of connective tissue (Hydroxyproline) in ground beef by autofluorescence spectroscopy. *Journal of Food Science* **64**: 377–85.