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Identification of Milk Types Using Front Face and Synchronous Scanning Fluorescence Spectroscopy

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FLUORESCENCE spectroscopy is a technique used to determine fluorescence spectrum that emits by fluorescent compounds. Milk has more than one fluorescent compounds such as tryptophan, vitamin A. Quick identification of milk types is needed for milk quality control and safety. The ability of two different fluorescence spectroscopy techniques (front face fluorescence spectroscopy; FFFS and synchronous scanning fluorescence spectroscopy; SFS) was determined to differentiate 40 milk samples according to their species (10 samples for each of buffalo's, cow's, goat's and sheep's milk). The statistical methods, principal component analysis (PCA) and factorial discriminant analysis (FDA) were used for better understanding the obtained results. FDA was applied separately on the first five principal components obtained from PCA which performed on the two different fluorescence techniques. Results obtained from FDA showed that 100% of correct classification was obtained for data sets from the two different fluorescence techniques. The obtained results confirmed that both the FFFS and SFS were capable of differentiating milk species.

Keywords: Milk, Synchronous fluorescence spectroscopy, Front-face fluorescence spectroscopy, Factorial discriminate analysis, Principal component analysis, Chemometric tools.

Introduction

Milk species identification has become an important issue in the dairy production with regard to milk authentication, to identify fraud and to assay quality control of milk and milk products. Discrimination of milk produced from different animal species requires measurement of a wide variety of constituents and detection of the minor differences among the samples (MacMahon et al., 2012 and Santos et al., 2013). This usually requires used the combined methods of several analytical techniques as electrophoretic techniques and chromatographic methods (Motta et al., 2014). These analytical methods are slow and expensive. Additionally, the dairy industry requires a rapid method for milk identification, detection of adulteration, and characterization of milk and dairy products (Hettinga et al., 2008, Nascimento et al., 2017).

An alternative to classic analytical methods, fluorescence spectroscopy coupled with chemometric tools has been applied effectively in several studies to identify different milk species (Kamal and Karoui, 2015 and Ullah et al., 2020). Fluorescence spectroscopy is a simple non-invasive and non-destructive technique, relatively inexpensive and allows rapid analysis. The fluorescence spectral features gave information about vitamin (A) located in fat globules, tryptophan residues in protein, riboflavin, and carotenoid components in milk samples which reflect the molecular structure of milk (Karoui and De Baerdemaeker, 2007 and Velioglu et al., 2017).

The two most common techniques of fluorescence spectroscopy are front face fluorescence spectroscopy (FFFS) and synchronous fluorescence spectroscopy (SFS).

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In FFFS technique, only excitation or emission wavelength has been used to determine one fluorophore component present in the samples. Protein tryptophan emission spectra and vitamin A excitation spectra were selected and recorded as indicators of milk identification. However, in SFS technique, both excitation and emission monochromators are scanned simultaneously, with a constant wavelength interval between them. SFS is very useful for the analysis of mixtures of fluorescent compounds, because both excitation and emission characteristics are included into a single spectrum. SFS exhibits results of several intrinsic fluorescent compounds (tryptophan, vitamin A, riboflavin and other aromatic amino acids) that present in food and provide the fingerprint spectrum of milk (Genis et al., 2019). In the literatures, there are many applications of fluorescence spectroscopy for the quality determination (Ntakatsane et al., 2011 and Velioglu et al., 2017) or identify of the dairy products (Herbert et al., 2000 and Boubellouta and Dufour, 2008).

Therefore, the aim of this research was to investigate the best technique of fluorescence spectroscopy with factorial discriminate analysis by which we can simply differentiate the different types of milk species. The samples were differentiated firstly by applying principal component analysis (PCA) to fluorescence spectral data of milk species and secondly by applying factorial discriminate analysis (FDA) to the first 5 principal components scores.

Materials and Methods

Milk samples

Fourty fresh milk samples (10 samples from each buffalo's, cow's, goat's and sheep's milk) were obtained directly from two different farms and transported immediately to the quality laboratory in airtight bottle for analysis. The milk samples were designated with single letters according to their type: Buffalo's (B), Cow's (C), Goat's (G) and Sheep's (S) milk.

Fluorescence spectroscopy measurements

Fluorescence spectra were obtained using a spectrofluorometer Model FP-6500 (JASCO Technologies, MD, USA) equipped with 15 W Xenon lamp source for excitation and a front-face geometry accessory adjusted to an angle of incidence of 56°. The fluorescence spectrum of tryptophan (305–400 nm) was recorded at excitation wavelength of 290 nm and emission wavelength range of 305-400 nm and for vitamin (A) was excited at 250–350 nm while emission wavelength was at 410 nm.

Synchronous fluorescence spectra were collected by simultaneously scanning the excitation and emission monochromators in the 250–600 nm range, with constant wavelength differences, $\Delta\lambda$ 80 nm, between them (Boubellouta and Dufour, 2010). Measurements were performed in triplicate.

Chemometric analysis

Spectral data were statistically analyzed by principal component analysis (PCA) and Factorial Discriminate Analysis (FDA) using SAISIR software package for MATLAB (MathWork, Natick, MA, USA). PCA provides graphical representations of similarities and differences in spectral data between samples by removing random variation and generating natural clustering within a data set (Cruz et al., 2013). Factorial Discriminate Analysis (FDA) was applied to the first 5 principal components of the PCA performed on the fluorescence spectral data in order to discriminate the milk samples (Karoui, 2004).

Results and Discussion

Fluorescence measurements

Milk exhibits strong fluorescence and several authors have proposed using fluorescence measurements to discriminate milk types from the recorded fluorescence spectra of intrinsic fluorophore in milk, in particular tryptophan residues in protein, vitamin A in milk fat and riboflavin (Velioglu et al., 2017).

Spectral difference of tryptophan

The tryptophan fluorescence spectra of the different milk species exhibited slight variations among milk types in the shape of the fluorescence spectra as shown in Fig.1. The tryptophan emission spectra displayed a maximum peak at a wavelength of 375 nm for buffalo's milk, 365 nm for cow's milk, 360 nm for goat's milk and 350 nm for sheep's milk. It is known that emissions of fluorophores are highly sensitive to their local environment; hence, the difference in physicochemical characteristics of milk samples influences variation in the shape of the fluorescence spectra (Dufour and Riaublanc, 1997).

Spectral difference of vitamin A

Figure 2 shows the vitamin A fluorescence spectra of the different milk species which slightly varied among milk types in the shape and intensity of the spectra. The shape of the vitamin A fluorescence spectra maxima located at 305 and 322 nm and a shoulder at 295nm. These results are agreement with Karoui and (Dufour, 2003) who reported that the shapes of the vitamin A excitation spectrum is correlated with the physical state of the triglycerides in the fat globules. In addition, the width of fluorescence spectra showed high differences among milk samples. Since, the width of fluorescence spectrum of cow's milk was larger than that of sheep's milk. This result may be considered as a fingerprint for the identification of milk species.

Synchronous fluorescence scan (SFS)

The SFS spectra reflect the specificity of intrinsic fluorophores and their microenvironments in the milk, whereas, they varied among different milk types. As illustrated from Fig. 3 the synchronous fluorescence spectra of the four milk

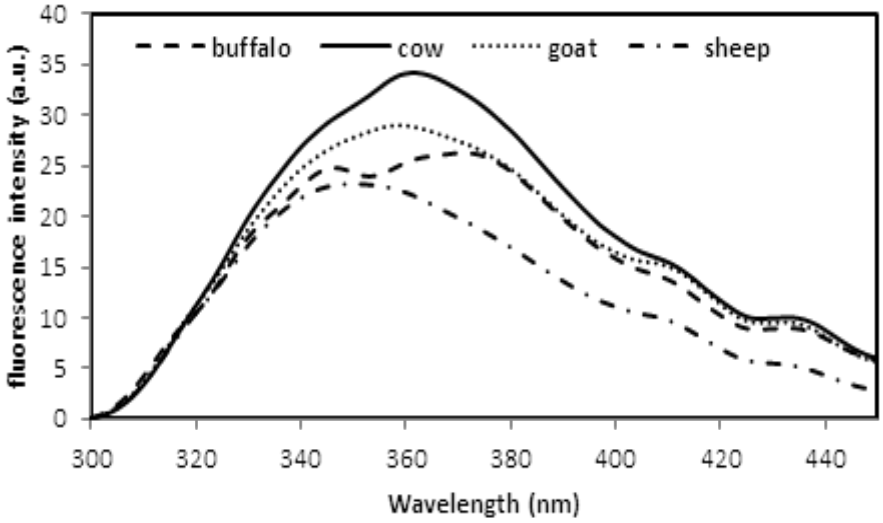

Fig. 1. Tryptophan emission spectra of buffalo's, cow's, goat's and sheep's milks recorded after excitation at 290 nm.

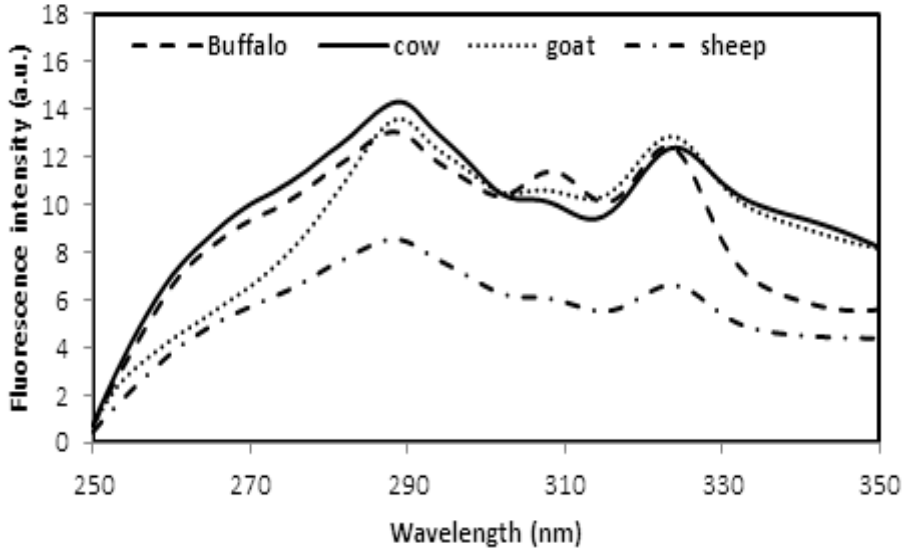

Fig. 2. Vitamin A excitation spectra of buffalo's, cow's, goat's and sheep's milks recorded after emission at 410 nm.

types located between 300-600 nm at $\Delta\lambda = 80$ nm. The SFS spectrum of the four milk species shows the fluorescence intensity variations at different wavelength positions. The main differences among milk species was observed in several spectral regions; 300–400 nm and 500-550nm which could be related to tryptophan, fat soluble vitamins and riboflavin, respectively. Emissions from tryptophan residues (first emission band) appear in the spectral region 300-390 nm and the wavelength positions of emission maxima of this fluorophore slightly varied between different milk types, whereas they located at 365 nm for cow's milk and 348 nm for sheep's milk. The tryptophan emission of sheep's milk was lower in intensity than that of other milk groups. Similar trend was observed with center position of the second emission band (vitamin A). The third band is largely attributed to riboflavin that can be considered as a fingerprint used to define milk species. An opposite trend is present with the third band (riboflavin). In this case maximal difference in band position was seen that it decreased from 530 nm for cow's milk nm to 525 nm for buffalo's milk, where the lower band was from buffalo's milk and upper band from cow's milk emission.

Chemometric results

In order to discriminate differences between the four milk species, PCA and FDA were applied to fluorescence spectral data.

Principal component analysis (PCA)

PCA is an exploratory method that shows similarities/dissimilarities among samples and thus facilitating understanding the dataset. PCA was applied separately to tryptophan, vitamin A and synchronous fluorescence data sets to visualise the distribution of milk samples according to milk species.

Milk identification from their tryptophan fluorescence spectra

Figure (4) shows the PCA score plots of different four milk based on tryptophan emission data. The PCA similarity map for tryptophan discriminated the samples according to PC_1 and PC_2 (which accounted for 84% of the total data variability). The PCA results indicated that cow's (negative PC_1 scores) and sheep's (positive PC_1 scores) milk samples clustered together on the upper side of the map, while the goat's and buffalo's milk samples on the bottom side of the map. According to PC_2, goat's milks had the highest negative scores, and buffalo's milks had the lowest negative scores.

Milk identification from their vitamin A fluorescence spectra

The similarity map defined by PC 1 and 2 of the PCA performed on the vitamin (A) fluorescence spectra is shown in Figure (5). This figure shows that cow's, goat's and buffalo's milk samples are located on the left side of the similarity map with negative scores; whereas the sheep's milk samples is located on the right side with positive scores according to PC_1. Considering the PC_2, goat's milk samples had positive score values, while buffalo's milk and cow's milk samples had negative score values.

Milk identification from their synchronous

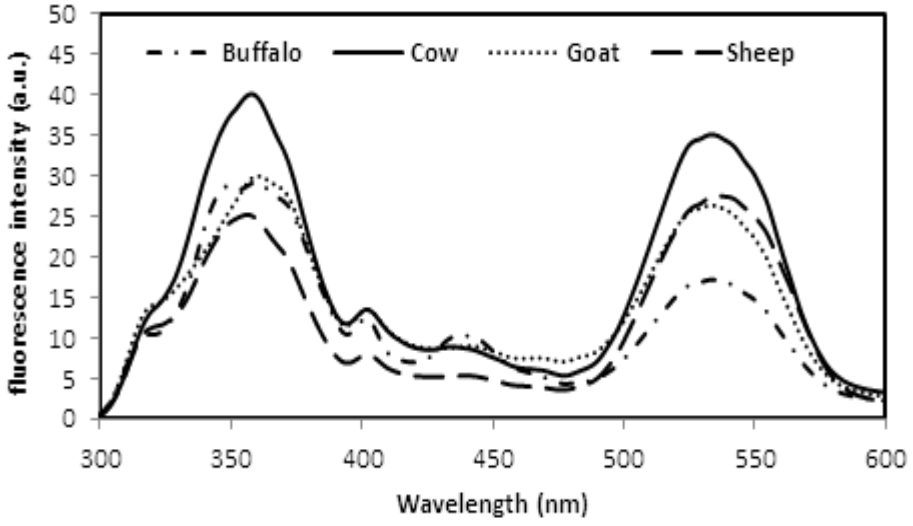

Fig. 3. Synchronous fluorescence spectra of buffalo's, cow's, goat's and sheep's milks at a wavelength interval of 80 nm.

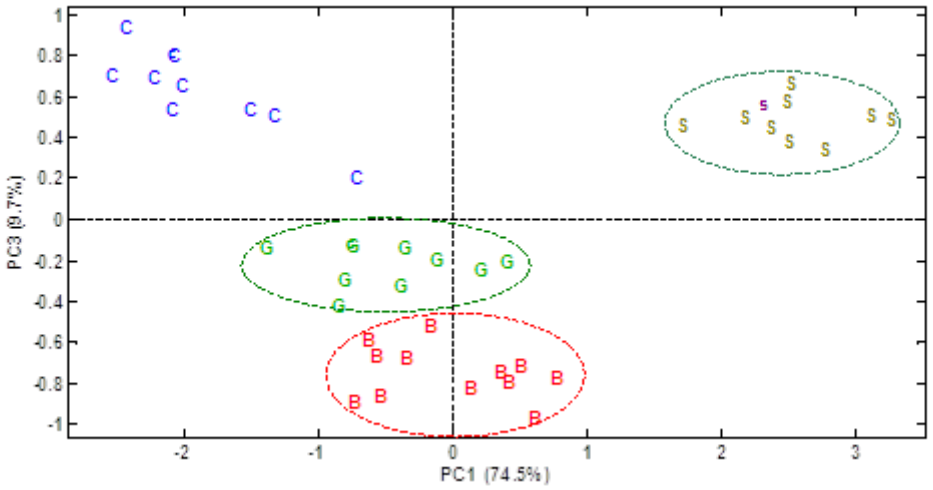

Fig. 4. Similarity map for the PC1 and PC2 of the PCA performed on the tryptophan emission fluorescence spectra of four milk species. Buffalo's (B), Cow's (C), Goat's (G) and Sheep's (S) milk.

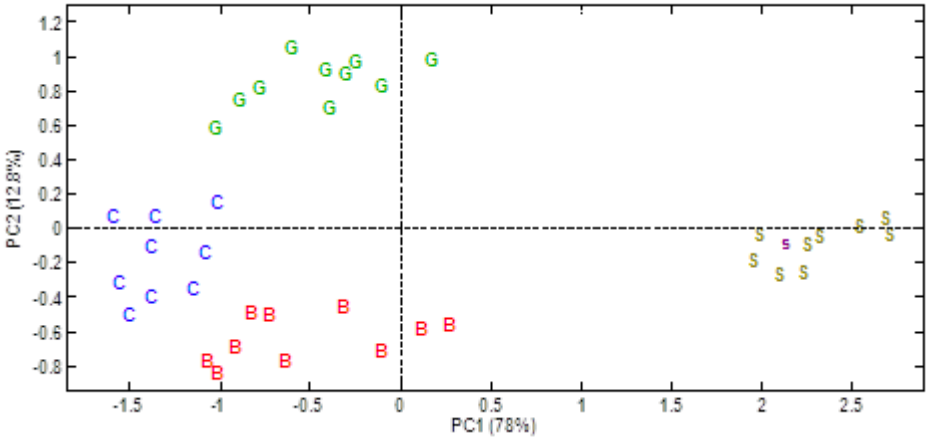

Fig. 5. Similarity map for the PC1 and PC2 of the PCA performed on the vitamin (A) excitation fluorescence spectra of milk species. Buffalo's (B), Cow's (C), Goat's (G) and Sheep's (S) milk.

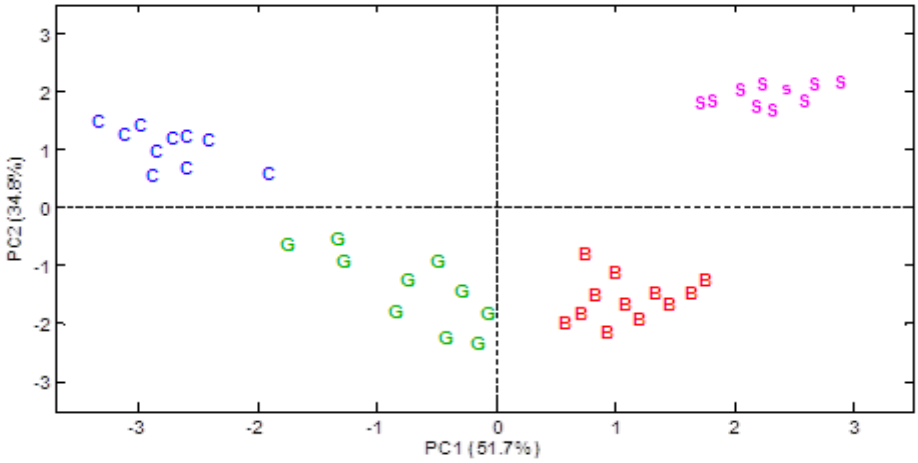

Fig. 6. Similarity map for the PC1 and PC2 of the PCA performed on the synchronous fluorescence spectra of milk species recorded at λ = 80 nm. Buffalo's (B), Cow's (C), Goat's (G) and Sheep's (S) milk.

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PCA similarity map defined by PC 1 and 2 performed on the synchronous fluorescence spectra of milk species is shown in Fig. 6. This figure shows a very good discrimination of samples according to milk type. The cow's with goat's milk; buffalo's with sheep's milk were displayed on the extreme positive and negative sides of the plot, respectively, according to PC_1. The PC_2 discriminate buffalo's milk samples located on the negative side from sheep's milk samples located on the positive side. This could be explained by similar physicochemical characteristic of the two milk types (buffalo and sheep's milk) in contrast to the other two milk types (cow's and goat's milk).

Factorial discriminant analysis (FDA)

The ability of fluorescence spectra data to differentiate between the four milk types was investigated by applying the FDA to the first 5 PCs of the PCA performed on the FFFS or SFS spectral data sets. Four groups (buffalo, cow, goat, and sheep) were created before performing FDA. The map defined by the discriminant factors 1 and 2 gave a clear discrimination of milk types (data not shown). Table (1) gives the classification results obtained from FDA for the four groups of milk species. It can be clearly seen in this Table that a 100% accurate classification was achieved for the two fluorescence techniques and all milk sample spectra were correctly matched within the four corresponding groups. Therefore, it was

TABLE 1. Classification results of FDA based on PCA scores for milk types fluorescence data.

Observed	Predicated				Total	% Correct Classification
	Buffalo's milk	Cow's milk	Goat's milk	Sheep's milk		
Fluorescence emission spectra of tryptophan						
Buffalo's milk	10	0	0	0	10	100%
Cow's milk	0	9	0	0	9	100%
Goat's milk	0	0	10	0	10	100%
Sheep's milk	0	0	0	10	10	100%
Total	10	9	10	10	39	100%
Fluorescence excitation spectra of vitamin A						
Buffalo's milk	10	0	0	0	10	100%
Cow's milk	0	9	0	0	9	100%
Goat's milk	0	0	10	0	10	100%
Sheep's milk	0	0	0	10	10	100%
Total	10	9	10	10	39	100%
Synchronous fluorescence spectral data						
Buffalo's milk	10	0	0	0	10	100%
Cow's milk	0	9	0	0	9	100%
Goat's milk	0	0	10	0	10	100%
Sheep's milk	0	0	0	10	10	100%
Total	10	9	10	10	39	100%

FDA: factorial discriminant analysis
PCA: principal component analysis

concluded that the fluorescence spectral features of milk types could be considered as fingerprints allowing a good identification of milk samples according to their milk species.

Conclusion

Results indicated that by measuring only a single fluorophore using FFS or several fluorescence components using SFS technique with FDA, it is possible to identify milk species with high specificity. The results show 100% correct classification rates for the four milk types. This appeared in the FDA, in which the groups of milk samples well separated from each other. Thus, front face fluorescence spectroscopy and synchronous fluorescence spectroscopy combined with the proper chemometric tools offers a promising approach for identifying the milk species.

References

Boubellouta T. and Dufour E. (2008) Effects of Mild Heating and Acidification on the Molecular Structure of Milk Components as Investigated by Synchronous Front-Face Fluorescence Spectroscopy Coupled with Parallel Factor Analysis. *Applied Spectroscopy,* **62** (5), 490-496.

Boubellouta T. and Dufour E. (2010) Cheese-Matrix Characteristics During Heating and Cheese Melting Temperature Prediction by Synchronous Fluorescence and Mid-infrared Spectroscopies. *Food and Bioprocess Technology,* 1-12.

Cruz A. G., Cadena R. S., Alvaro M. B., Sant'Ana A. d., Oliveira C. A., Faria J. D., Bolini H. and Ferreira M. (2013). Assessing the use of different chemometric techniques to discriminate low-fat and full-fat yogurts. *J LWT-Food Science Technology,* **50** (1), 210-214.

Dufour E. and Riaublanc A. (1997) Potentiality of Spectroscopic Methods for the Characterisation of dairy Products. II. Mid Infrared Study of the Melting Temperature of Cream Triacylglycerols and of the Solid Fat Content in Cream. *Le lait,* **77**, 671-681.

Genis D. O., Bilge G., Sezer B., Durna S. and Boyaci I. H. (2019). Identification of cow, buffalo, goat and ewe milk species in fermented dairy products using synchronous fluorescence spectroscopy. *Food Chemistry,* **284**, 60-66.

Herbert S., Mouhous-Riou N., Devaux M. F., Riaublanc A., Bouchet B., Gallant D. J. and Dufour É. (2000) Monitoring the identity and the structure of soft cheeses by fluorescence spectroscopy. *Le Lait,* **80** (6), 621-634.

Hettinga K. A., Van Valenberg H. and Van Hooijdonk A. (2008). Quality control of raw cows' milk by headspace analysis. *International Dairy Journal,***18** (5), 506-513.

Kamal M. and Karoui R. (2015). Analytical methods coupled with chemometric tools for determining the authenticity and detecting the adulteration of dairy products: A review. *Trends in Food Science & Technology,* **46** (1), 27-48.

Karoui R. (2004). Contribution à l'étude des propriétés rhéologiques et à la détermination de l'origine géographique des fromages aux moyens des méthodes spectroscopiques et chimiométriques. page 118. Université Blaise Pascal, Clermont-Ferrand.

Karoui R. and Baerdemaeker J. D. (2007) A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry,* **102** (3), 621-640.

Karoui R. and Dufour É. (2003) Dynamic testing rheology and fluorescence spectroscopy investigations of surface to centre differences in ripened soft cheeses. *International Dairy Journal,* **13** (12), 973-985.

MacMahon S., Begley T. H., Diachenko G. W. and Stromgren S. A. (2012) A liquid chromatography–tandem mass spectrometry method for the detection of economically motivated adulteration in protein-containing foods. *Journal of Chromatography* A, **1220**, 101-107.

Motta, T. M., Barreto R. B., Andrade R. B., Lorenzini D. M., Meneghini L. Z. and Pizzolato T. M. (2014) Detection and confirmation of milk adulteration with cheese whey using proteomic-like sample preparation and liquid chromatography–electrospray–tandem mass spectrometry analysis. *Talanta,* **120**, 498-505.

Nascimento C. F., Santos P. M., Pereira-Filho E. R. and Rocha F. R. (2017) Recent advances on determination of milk adulterants. *Food Chemistry,* **221**,1232-1244.

Ntakatsane M. P., Yang X. Q., Lin M., Liu X. M. and Zhou P. (2011) Short communication: Suitability of fluorescence spectroscopy for characterization of commercial milk of different composition and

origin. *Journal of Dairy Science,* **94** (11), 5375-5380.

Santos P., Pereira-Filho E., and Rodriguez-Saona L. (2013). Rapid detection and quantification of milk adulteration using infrared microspectroscopy and chemometrics analysis. *J Food chemistry,* **138** (1), 19-24.

Ullah R., Khan S., Ali H. and Bilal M. (2020). Potentiality of using front face fluorescence spectroscopy for quantitative analysis of cow milk adulteration in buffalo milk. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* **225**, 117518.

Velioglu S. D., Ercioglu E. and Boyaci I. H. (2017) Rapid discrimination between buffalo and cow milk and detection of adulteration of buffalo milk with cow milk using synchronous fluorescence spectroscopy in combination with multivariate methods. *Journal of Dairy Research,* **84** (2), 214-219.

توصيف أنواع اللبن بإستخدام طريقتين مختلفتين للتحليل الفلوروسينتي

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التحليل الفلوروسينتي عبارة عن قياس الطيف الفلوروسينتي المنبعث من بعض المركبات التي لها طيف فلوروسينتي. ويحتوي اللبن على العديد من تلك المركبات مثل (التربتوفان وفيتامين A وغيرها). وتحتاج مصانع الألبان إلى طرق سريعة لتعريف وتوصيف أنواع اللبن المختلفة لكشف الغش والتحكم في الجودة والأمان. ومن هنا نجد أن الهدف الرئيسي لهذا البحث هو إختبار قدرة جهاز التحليل الفلوروسينتي بطريقتين مختلفتين (front face fluorescence spectroscopy FFS) و synchronous fluorescence spectroscopy (SFS) على تمييز ٤٠ عينة من أنواع اللبن المختلفة (١٠ عينات من كل من اللبن الجاموسي ، اللبن البقري ، لبن ماعز و لبن أغنام). مع أستخدام بعض الطرق الإحصائية المتقدمة مثل (factorial discriminant analysis FDA و principal component analysis PCA) لتوضيح النتائج المتحصل عليها من الجهاز بطريقة أفضل. ولقد أظهرت النتائج المتحصل عليها من PCA و FDA أنه يمكن تمييز أنواع اللبن المختلفة بنسبة ١٠٠٪ وذلك من البيانات المأخوذة من جهاز القياس الفلوروسينتي. وبالتالي فقد أكدت النتائج المتحصل عليها أن جهاز القياس الفلوروسينتي بطريقتيه المختلفتين FFS و SFS قادر على تمييز أنواع اللبن المختلفة.