Journal of Advanced Research 15 (2019) 19–25

Contents lists available at ScienceDirect

Journal of Advanced Research

journal homepage: www.elsevier.com/locate/jare

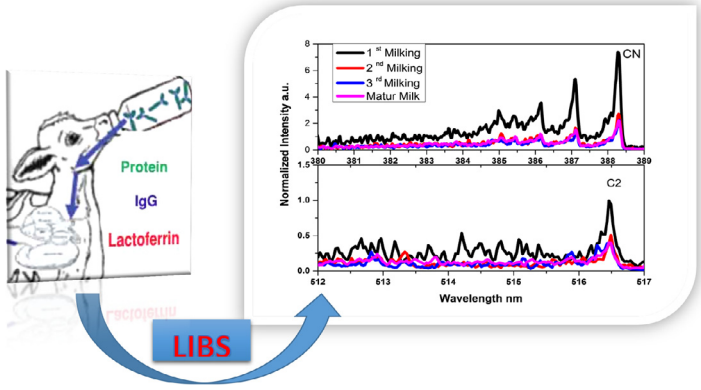
Original Article

Evaluation of proteins in sheep colostrum via laser-induced breakdown spectroscopy and multivariate analysis

Z.A. Abdel-Salam [a], S.A.M. Abdel-Salam [b], I.I. Abdel-Mageed [b], M.A. Harith [a,*]

[a] National Institute of Laser Enhanced Science (NILES), Cairo University, Giza, Egypt
[b] Faculty of Agriculture, Department of Animal Production, Cairo University, Giza, Egypt

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 16 April 2018
Revised 25 June 2018
Accepted 4 July 2018
Available online 6 July 2018

Keywords:
Sheep colostrum
Protein
Lactoferrin
LIBS
PCA

ABSTRACT

Colostrum is essential to guarantee normal and healthy feeding in newborn ruminants during the first hours. In the present work, Laser-Induced Breakdown Spectroscopy (LIBS), as a spectrochemical analytical technique, and principal component analysis (PCA) as a multivariate analysis method were used to evaluate colostrum compared to mature milk of sheep to plan the nutritional strategies for newly born lambs. Samples of colostrum have been collected from thirty-three Barki ewes. The sheep were milked every 12 h three times after birth, the fourth sample of mature milk is taken from milking in the 7th day postpartum. The spectrochemical analytical results depicted that the intensities of CN and C_2 spectral bands, and C 247.86 nm atomic line (as an indicator for protein content in LIBS spectra) are higher in colostrum than that in milk. This relationship has been confirmed by measuring the total protein in the same samples conventionally. The relation between calcium and protein percentage has also been demonstrated. Moreover, it has been shown that the higher is the CN bands' intensity the lower is the bacteria count in colostrum samples, owing to the high levels of lactoferrin with its antibacterial effect. The qualitative analysis of LIBS data using PCA led to a pronounced discrimination between colostrum and mature milk. The present study demonstrates that it is, in principle, possible to make use of the analytical and chemometric results in dairy farms to evaluate sheep colostrum to manage the nutritional strategies for the lambs.

Peer review under responsibility of Cairo University.

* Corresponding author.
E-mail address: mharithm@niles.edu.eg (M.A. Harith).

https://doi.org/10.1016/j.jare.2018.07.001
2090-1232/

Introduction

Colostrum is the milk secreted postpartum in ruminants. It differs from mature milk both in color and composition. Good quality colostrum has a thick consistency and yellowish color. In the early stages after birth; colostrum is rich in proteins, vitamins, immunoglobulins. [1,2], lactoferrin [3], fat, and minerals [4]. The milk composition turns gradually to its normal status from the second to approximately the eights milking; the milk secreted during this period is identified as transit milk. The transition from colostrum to mature milk may take around 72 h [5,6]. Colostrum contains a high level of several nutrients that are important for lamb health and performance. It also contains a high level of antibodies against a variety of infectious agents. Newborn lambs haven't any antibodies because the placenta prevents the transfer of antibodies from the mother ewe to the fetus.

Colostrum plays indispensable immunization role, leading to direct vital beneficial effects on the newborn endocrine and metabolic systems. In addition, colostrum is a source of energy necessary to provide the lamb with heat needed to fight hypothermia [7]. The laxative effect of colostrum is also very important since it helps the young animal in getting rid of meconium from the intestines [8]. The failure of young animals in acquiring passive immunity leads to higher incidence of microbial infections and consequently expected higher mortalities [9]. Ahmad et al. reported significantly higher concentrations of immunoglobulin in lambs that have been survived after the neonatal period than lambs which died during the same period [10]. Moreover, it has been found that for ewes that produce low quality colostrum with lower IgG content, their lambs have had noticeably higher mortality rates [11]. Recently, there is a remarkable increase in the number of dairy-sheep farms globally. Especially in developing countries, lambs breeding proceeds under artificial feeding regimes to quantitatively and qualitatively raise the production of sheep milk. In view of what mentioned above, major changes in sheep milk taking place during lactation are mainly in the colostrum composition [1,2].

Laser-induced breakdown spectroscopy (LIBS) is a spectrochemical analytical technique that has been reported in the literature more than fifty years ago [12]. This technique depends on the spectroscopic detection and analysis of atomic, ionic and, in some cases, molecular emission of a laser produced plasma. As an analytical technique, LIBS possess unique capabilities, namely it can be used to analyze solids, liquids, and gases, it needs no or very little sample preparation, it can analyze materials of low as well as high atomic numbers, it can perform simultaneous multi-elemental detection, it can be used for *in situ* and real-time measurements, it is also relatively very fast and it is cost-effective [13,14]. In the last two decades, LIBS has been used in numerous applied fields, especially in the composition analysis of biological samples [15–17]. Although of the fact that LIBS is essentially an elemental analysis technique, but it has been also used for the detection and analysis of small molecules such as CN, C_2, and OH, especially in organic and biological materials [18]. In case of milk samples, LIBS has been used to assess proteins and organic materials by monitoring the CN and C_2 bands in the spectra of such samples [13,19].

In the present work, the CN and C_2 spectral bands, as well as the carbon atomic line at 247.86 nm in the LIBS spectra of sheep colostrum samples are exploited in the qualitative evaluation of the proteins contents in such samples. The CN and C_2 molecular bands in the LIBS spectra are followed up in different milking days and the relation between proteins concentration, as a function of the molecular bands' intensity, and calcium spectral lines intensity, as well as the microbial count, has been also demonstrated. Because of the complexity of LIBS spectra, it was necessary to make use of multivariate data analysis to obtain significant information from a large number of collected spectra. Therefore, LIBS results have been corroborated by multivariate data analysis via unsupervised pattern recognition technique, namely principal component analysis (PCA). Using portable LIBS system and proper statistical software for the reliable evaluation of proteins in colostrum will help in establishing feeding strategies of lambs in animal production farms.

Material and methods

Colostrum and milk samples

Samples of colostrum have been collected from thirty-three Barki ewes in the sheep farm located in agricultural research and experimental station, Faculty of Agriculture, Cairo University, Egypt.

Generally, the chosen animals were healthy and did not receive any medications before or during the experimental period. Postpartum, lambs were fed by artificial rearing, and the sheep were milked every 12 h for the first three days after birth, the fourth sample is taken from milking in the 7th day (i.e. 3 consecutive colostrum samples and one mature milk sample from each animal). After bacteriological analysis, the collected colostrum and milk samples (300 mL each) were frozen at -20°C until spectroscopic analysis time.

Directly before performing the spectroscopic measurements, the frozen samples were thawed in tap water path. One droplet of each sample (0.5 mL/droplet) was distributed onto high-quality ashless filter paper and left for about 15 min in a clean atmosphere to partially dry and homogeneously expand onto the filter paper.

LIBS arrangement

A typical LIBS experimental setup, described in details elsewhere [20], has been used in the present work. Briefly, the used laser was a Q-switched Nd: YAG laser (Brilliant Eazy, Quantel, France) producing 5 ns laser pulses each of 150 mJ energy at 1064 nm wavelength and 1 *Hz* repetition rate. The laser beam was focused onto the sample by means of a planoconvex quartz lens (f = 10 cm). The sample under investigation was mounted on an X-Y micrometric translation stage. The delay time was 2 μs and the gate width 2 μs. A 2 m long fiber-optic cable of 600 μm core diameter is used to gather and guide the emission light of the laser produced plasma to the spectrometer entrance slit. An echelle spectrometer (Mechelle 7500, Multichannel, Sweden) coupled to a gateable ICCD camera (DiCAM-Pro, PCO computer optics-Germany) has been used for dispersion and detection of the collected plasma emission light. The ICCD and the spectroscopic system cover a wide spectral range from 200 nm up to 900 nm. Each measured spectrum represents the average of 25 spectra obtained as 5 spectra from each of five fresh samples spots on the dried droplet on the ashless filter paper. LIBS^{++} software has been used for data acquisition, further identification, and analysis of the spectral lines in the obtained LIBS spectra.

Colostrum sampling and bacteriological analysis

Colostrum was collected by the dairy technician with a 10 mL sterilized syringe from the nursing bottle directly before the first lactation of the newborn lambs. Date, as well as lamb and sheep identification numbers, are given to each sample. After bacteriological analysis, samples have been directly frozen and stored at -20 °C before starting the conventional spectroscopic analysis.

The samples under investigation were assessed bacteriologically using the Standard Plate Count (SPC) according to [21,22].

Laboratory determination of total protein

Total protein content from samples of 16 animals chosen randomly was determined by routine laboratory procedures using an automated infrared milk analyzer (MilkoScan FT1, FOSS, Demark) at the central laboratory of Faculty of Agriculture, Cairo University.

Statistical analysis

ANOVA procedure was used to estimate the effect of animals and day of milking on the CN, C_2 bands in the LIBS spectra as indicators of total protein using SPSS IBM SPSS Statistics. A descriptive statistical analysis was used to estimate means and standard deviation of different variables for every 4 milking days. The Duncan procedures were used to test the significant differences between the least square means of the day of milking (first to fourth day postpartum) [23].

The statistical model used to study the effect of day and animal on the studied variables was:

$$Y_{ijk} = \mu + A_i + D_j + A_i(D_j) + e_{ijk}$$

where

Y_{ijk} = the observation of i^{th}...

μ = overall mean,

A_i = the fixed effect of ith animal (i = 1–33),

Dj = the fixed effect of jth day of milking (j = 1–4),

A_i * D_j = the interaction between ith animal and jth day of milking,

and e_{ijk} = random effect of residual.

The fixed effects of animal (A_i) and day of milking (D_j) and the interaction between them (A_i * D_j) was highly significant ($P < 0.01$) in the model used on the studied variables.

The standard deviation of the experimental results has been calculated and used to set the error bars in the figures.

For chemometric analysis, PCA has been applied as a multivariate analysis method. PCA furnishes a fast and simple technique to analyze the distribution of the spectral data. Origin Pro-2017 software (OriginLab Corporation, MA, USA) has been used for PCA analysis of the obtained LIBS spectra and factor score plots have been used to discriminate between colostrum and mature milk from the relevant LIBS spectra. To enhance the discrimination performance of the PCA, a priori reduction of the variables has been done by limiting the wavelength ranges only to regions that include spectral lines relevant to the distinction procedure.

Results

As mentioned above, colostrum intake is a key factor for newborn ruminant survival because the placenta does not allow the transfer of immune components. Therefore, newborn ruminants depend entirely on passive immunity transfer from the mother to the neonate, through the suckling of colostrum.

To follow up the relative concentration of proteins in the samples under investigation, the intensity of three CN spectral bands and one C_2 band, normalized for the strong and well-resolved calcium line at 422.67 nm, have been studied. In fact normalization of the spectra was important to avoid probable experimental fluctuations. In the normalization procedure, the peak values of the spectral bands and lines were used. For CN; the elected emission bands were at 386.16 nm, 387.14 nm, and 388.34 nm while the C_2 band was at 516.52 nm. These bands have been selected because of being well resolved, and free of spectral interference.

Fig. 1(upper) depicts a comparison between the CN bands intensities in the UV region for sheep colostrum and milk within the first few milking days. The spectra demonstrate the high protein content in the colostrum compared to mature milk. Fig. 1 (lower) shows similarly the behavior of C_2 emission band at 516.52 nm in colostrum and milk, the inset depicts the same comparison for the carbon atomic line at 247.86 nm. Fig. 2 demonstrates the decrease in the intensities of the calcium spectral lines at 393.33 nm, and 396.84 nm with the milking days.

Fig. 3 depicts the normalized intensity of 825 spectra (33 samples × 5 laser shots × 5 fresh spots) for CN, and C_2, for consecutive milking, the error bars represent the standard deviation of the experimental data. Different letters (a, b) indicate significant differences ($P < 0.05$). The intensity values of (CN) and C_2 bands were increased significantly ($P < 0.05$), at first, second and third milking compared with the milk samples as shown in Fig. 3.

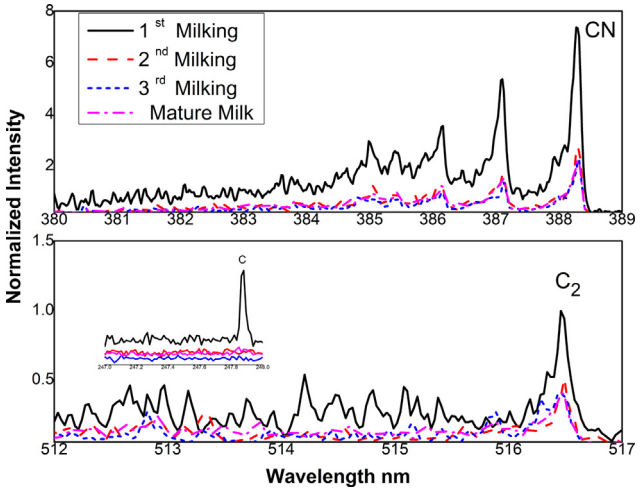


Fig. 1. Comparison between the violet CN band (upper), the swan C_2 band (lower), and C 247.86 nm (inset) in sheep colostrum and milk, at four different milking times in the average of 825 LIBS spectra.

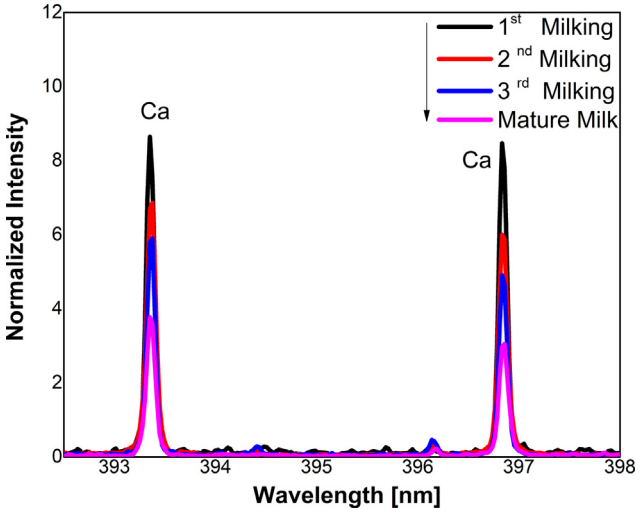


Fig. 2. Comparison between the calcium intensity of sheep colostrum and milk, at four different milking times in the average of 825 LIBS spectra.

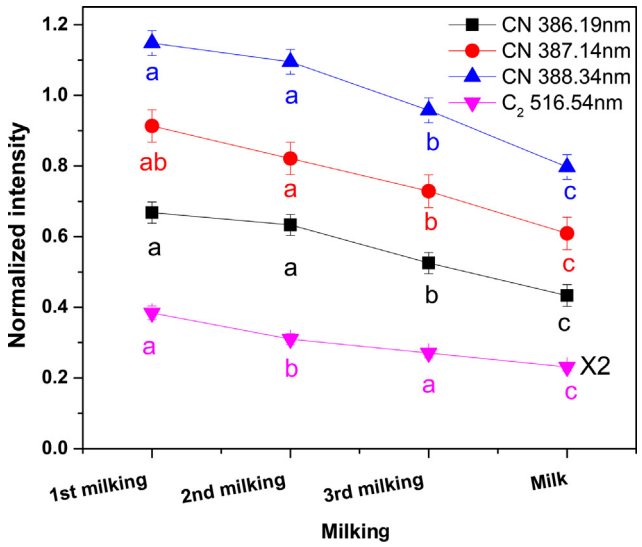


Fig. 3. The trend of the intensity changes of the CN and C_2 in sheep colostrum and milk, at four different milking times. The error bars represent the standard deviation of the experimental data. Different letters (a, b and c) indicate significant differences ($P < 0.05$).

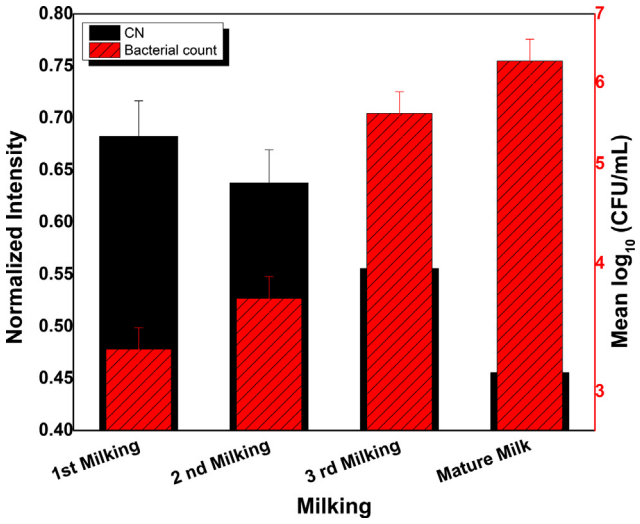


Fig. 5. Bar graph of the normalized intensity of CN and total bacterial counts $\log_{10}$ (CFU/mL) versus different milking times of the same samples under investigation.

Fig. 4 shows clearly that the CN and C_2 intensities increase with increasing protein content. In addition to CN and C_2, this figure depicts also the relation between the total protein concentration and the calcium content in the different sheep milking. The atomic line of carbon 247.86 nm shows a typically similar trend (not shown in the figure).

A bar graph of the normalized intensities of CN band and the total bacterial count $\log_{10}$ CFU/mL versus different milking is shown in Fig. 5. The results obtained demonstrate that the higher is the CN intensity the lower is the total bacterial count in colostrum and milk samples and vice versa. In Fig. 6, the normalized intensity of the CN band at 388.34 nm has been plotted versus the total proteins concentrations measured conventionally for 16 samples chosen randomly from the overall 33 samples. The plot revealed a very good linear relation with $R^2 = 0.96$.

Application of PCA on the obtained LIBS spectral data

As mentioned above, the obtained LIBS spectra were statistically processed with the PCA chemometric method. To reduce the number of variables, and consequently improve the discrimination procedure of the used multivariate analysis technique, the analyzed LIBS spectral data was restricted to three ranges of the wavelength. The first wavelength range was from 200 nm to 250 nm, covering the carbon 247.86 nm atomic line, the second is from 385 nm to 390 nm, which includes the CN bands (386.15, 387.12 and 388.31 nm), while the third range was from 392 nm up to 431 nm, covering many major calcium lines (393.37, 396.85, 422.67, 428.3, 429.89, 430.77 nm, . . . etc.). To improve the discrimination of the PCA technique, the three spectral ranges have been merged. Fig. 7 (upper) depicts the PCA score plot results which demonstrate the distinction between colostrum and mature milk adopting the whole spectral range. While Fig. 7 (lower) shows the PCA plot for the merged three spectral ranges.

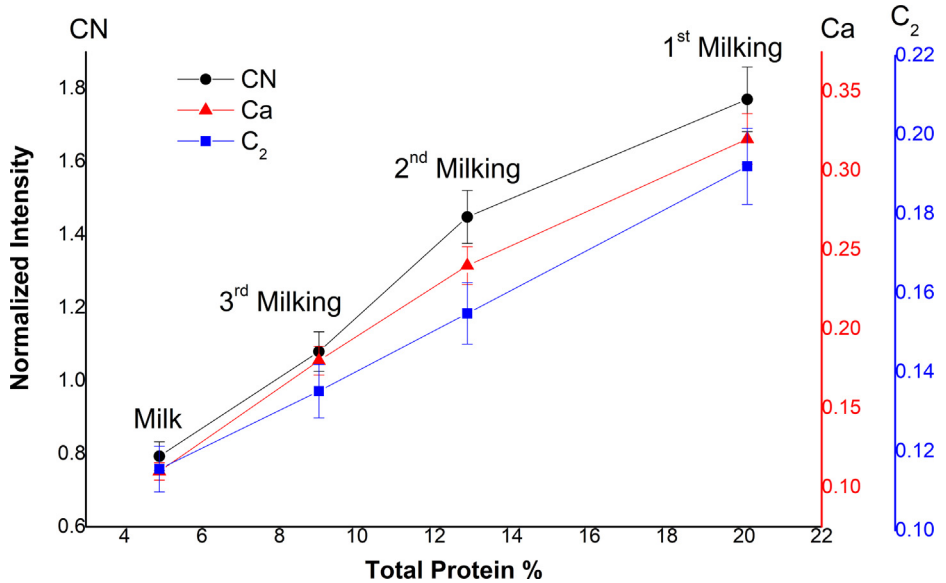


Fig. 4. Trends of normalized intensity values for CN, C_2 emission bands and Ca line for sheep colostrum and milk at different milking times versus total protein (%).

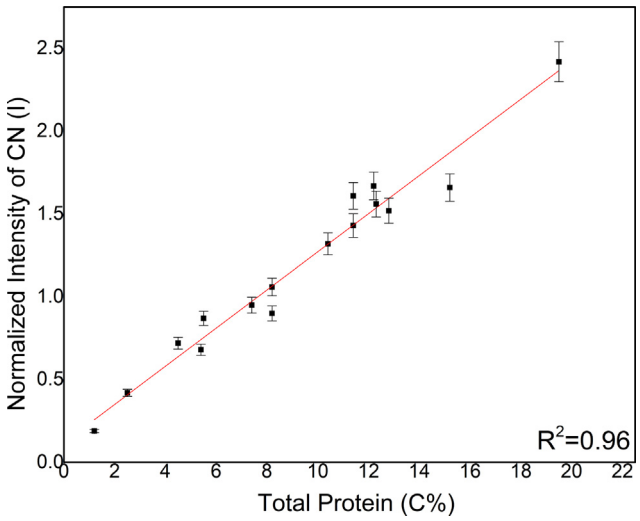


Fig. 6. Correlation curve for CN normalized intensity values versus the total protein (%). The solid line is the linear fitting of the experimental points [C (%) = 0.1152 I + 0.1198]. The error bars are the standard deviation of the data.

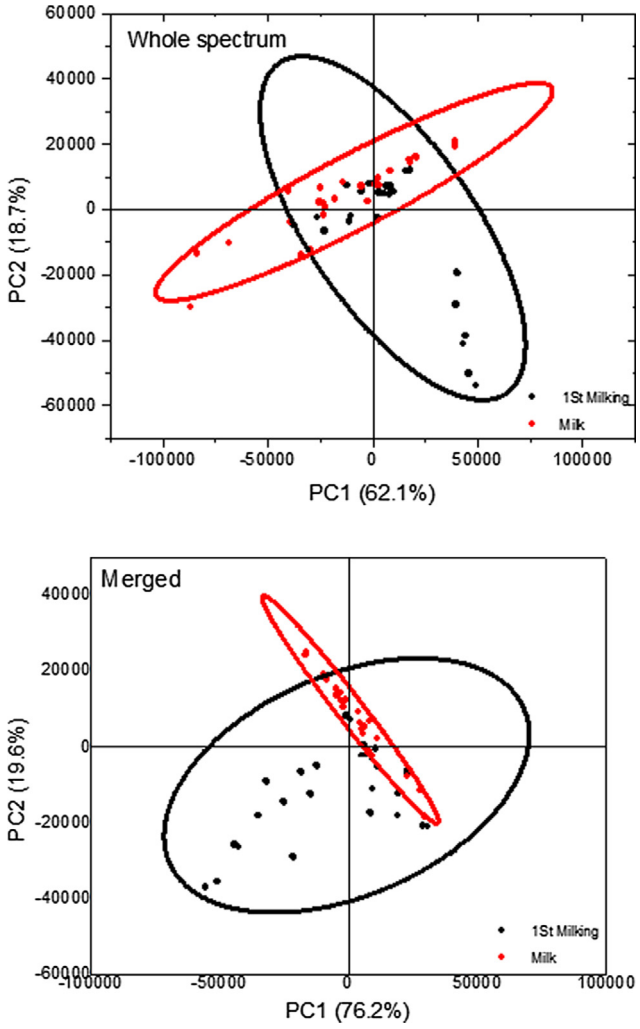


Fig. 7. PCA analysis for colostrum and milk of sheep for the whole spectral range 200 nm to 750 nm (upper), and merging the three spectral ranges 200–250 nm, 385–390 nm, and 392–431 nm (lower).

Discussion

CN and C_2 molecular bands, as well as one carbon spectral line, which are relevant to the organic contents in the samples, have been chosen to be followed up in LIBS spectra in view of the previously published works [6,13,15,19]. It is well known that CN and C_2 molecular bands in LIBS spectra can be used in many kinds of research to detect and monitor some molecules containing carbon and nitrogen such as proteins [6,13,17]. Therefore, it is possible to evaluate proteins in milk through the presence of CN, and C_2 bands as well as the carbon line in the LIBS spectra of both colostrum and milk samples. The present results indicated that CN and C_2 emission bands have a higher intensity in colostrum than in mature milk samples as shown in Fig. 1. In fact, LIBS results cannot differentiate between different types of protein, but it can be useful in evaluating the total protein in colostrum and mature milk samples considering CN and C_2 relative intensities in relevant spectra [1,2]. Similarly, the relative intensity of carbon atomic lines in the LIBS spectra can be used in combination with CN and C_2 bands, as indicators of the proteins in the investigated colostrum and milk samples (see the inset in the lower Fig. 1). Within the first hours after parturition, the colostrum contains high concentrations of protein substances, especially, immunoglobulins. In fact, it has been found that the content of free peptides and amino acids are high in the colostrum of all mammals [24]. In the current study, the intensity values of CN and C_2 as indicators of protein content within the first three days (first, second and third milking) after postpartum and the mature milk (in the 7th day) are shown in Fig. 3. Significantly high values of the protein concentrations show up in colostrum (1st milking) followed by lower values for the second, third milking and finally mature milk.

To validate the LIBS results, total proteins have been measured conventionally for all samples. Fig. 4 shows the proportionality between the CN and C_2 intensities in the LIBS spectra and the total protein content. This supports the use of such molecular bands as indicators of the protein in the investigated samples. On the other hand, casein which is a major protein component in milk is accompanied by a high concentration of calcium [25]. In the same Fig. 4, this correlation between total protein (including casein) and calcium content in the colostrum and milk samples has been clearly demonstrated.

According to Roig et al. [25] and Thapa [26] immunoglobulins (IgG) are essentially required for newborns to ensure the specific antimicrobial activity (mainly to prevent probable infections). It is clear that transfer of immunoglobulins to lambs through colostrum takes place directly after parturition. In the present study, it has been shown (Fig. 5) that, the higher is the CN intensity the lower is the total bacterial count in colostrum and sheep milk samples and vice versa. It should be mentioned that colostrum and milk contain high levels of lactoferrin which has inhibition effects on bacteria, viruses, and parasites [27–29]. The very high affinity of lactoferrin for iron is relevant to its function against such microorganisms. In fact, lactoferrin can be considered as part of the immune system because of its effects on pathogens growth [30]. This antibacterial mechanism justifies clearly the results depicted in Fig. 5. It is, in principle, possible to determine the concentration of the proteins in any colostrum or milk sample by measuring the normalized intensity of the CN band in its LIBS spectrum. This can be done by making use of the linear relation depicted in Fig. 6 between the CN normalized intensity and the corresponding proteins concentration measured conventionally. Using a portable LIBS system it is, of course, possible to evaluate the protein in colostrum or milk samples *in situ*, i.e. in dairy or animal production farms for example.

Principal component analysis (PCA) can be exploited for the identification of similarities and dissimilarities in measured data. Consequently, it is possible to utilize the factor score plots for the elucidation of similar or different experimental data. The results shown in Fig. 7 demonstrate that the spectral data collected from LIBS measurements combined with PCA as a chemometric method could become an interesting tool to evaluate sheep colostrum. Fig. 7 confirms the spectroscopic results and indicates that the changes in the composition, either in the protein (including casein, lactoferrin ... etc.) or in the calcium content, can be exploited by the PCA multivariate statistical approach to distinguish between colostrum and mature milk. PCA led to a good discrimination, in Fig. 7 (upper), PC1 and PC2 represent 80.8% of the total variance for the whole spectral wavelength range (200–750 nm) with PC1 = 62.1% & PC2 = 18.7%. However, merging three spectral ranges, 200 nm to 250 nm including the carbon line, 385–390 nm covering the CN band, and 392 nm up to 431 nm which contains many calcium spectral lines, led to a pronounced improvement in the discrimination between colostrum and milk where the principal components represent 95.8% of the total data variance with PC1 and PC2 equal 76.2% and 19.6% respectively (Fig. 7 lower). It is clear that exploiting the merged three wavelength ranges in PCA is superior in the discrimination between colostrum and milk than PCA using the whole spectral wavelength range,

However, in view of the obtained results, LIBS as spectrochemical analytical technique, combined with multivariate analysis using PCA can be considered as a promising, fast, reliable and accurate approach for real-time and *in situ* evaluation of colostrum and milk. It should be also mentioned here, that laser-induced fluorescence (LIF) has been used successfully in a previous work to evaluate colostrum [31]. This demonstrates that both LIBS and LIF are privileged available spectrochemical analytical techniques for the evaluation of colostrum and milk.

Conclusions

In conclusion, this study demonstrated that spectrochemical and multivariate analysis can be used successfully for evaluating proteins in sheep colostrum. Compared to the conventional techniques used in similar studies, LIBS is fast, safe, simple and can be used *in situ*. Proteins have been evaluated using the molecular bands of CN and C_2 as well as the carbon line at 247.86 nm in the LIBS spectra of colostrum and milk. The resemblance of calcium and proteins trends in sheep milking samples has been also demonstrated using LIBS. In addition, it has been shown that higher proteins concentration means higher lactoferrin and consequently lower bacterial count. The proportionality between the normalized intensity of the CN band and the concentration of the proteins can be used for the quantification of proteins in any unknown colostrum/milk sample. The multivariate analysis of the obtained analytical data using PCA provided satisfactory discrimination between colostrum and mature milk. This result has been reached in cases of using the spectral ranges including the carbon line and CN bands or that encompassing the calcium spectral emission lines. The experimental approach using typical LIBS setup is simple, fast and needs no or very little sample preparation. Besides, the PCA multivariate technique is also trustworthy, simple and does not need complicated statistical calculations. It is, in general, possible to make use of portable LIBS system and proper software for PCA in dairy farms to evaluate sheep colostrum in order to plan the nutritional strategies for the lambs.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

References

[1] Csapo JJ, Keszthelyil T, Csapo-Kiss Z, Lengyel A, Andrassy- Baka B, Varga-Visi E. Composition of colostrum and milk of different breeds of ewes. Acta Agraria Kaposvariensis 1998;2:1–21.
[2] El-Hommosi FF, Abd El-Ati MN, Mousa MT, Hamdon HAM, Shelab MM, El-Tahlawy R, et al. Quantitative studies in some colostrum and blood components of Chios and Egyptian Oasis (Farafra) sheep. Eighth Sci Congr 1998:558–73.
[3] Abd-El-Gawad IA, El-Sayed EM, Mahfouz MB, Abd-El-Salam AM. Changes of lactoferrin concentration in colostrum and milk from different species Egypt. J Dairy Sci 1996;24:297–308.
[4] Hadjipanayiotou M. Composition of ewe, goat and cow milk and of colostrum of ewes and goats. Small Ruminant Res 1995;1995(18):255–62.
[5] Campana WM, Baumrucker CR. Hormones and growth factors in bovine milk. In: Jensen RG (Eds.), Handbook of Milk Composition. San Diego, Academic Press; 1995. P. 467–494.
[6] Abdel-Salam Z, Abdelghany S, Harith MA. Characterization of milk from mastitis-infected cows using laser-induced breakdown spectrometry as a molecular. Analytical technique. Food Anal Methods 2017;10:2422–8.
[7] Rauprich ABE, Hammon HM, Blum JW. Influence of feeding different amounts of first colostrum on metabolic, endocrine and health status and on growth performance in neonatal calves. Anim Sci J 2000;78:896–908.
[8] Pattinson SE, Davies DAR, Winter AC. Changes in the secretion rate and production of colostrum by ewes over the first 24 h postpartum. Animal Sci 1995;61:63–8.
[9] Hashemi M, Zamiri MJ, Safdarian M. Effects of nutritional level during late pregnancy on colostral production and blood immunoglobulin levels of Karakul ewes and their lambs. Small Ruminant Res 2008;75:204–9.
[10] Ahmad R, Khan A, Javed MT, Hussain I. The level of immunoglobulins in relation to neonatal lamb mortality in Pak-Karakul sheep. Vet Arhiv 2000;3:129–39.
[11] Tabatabaei S, Nikbakht G, Vatankhah M, Sharifi H, Alidadi N. Variation in colostral immunoglobulin G concentration in fat tailed sheep and evaluation of methods for estimation of colostral immunoglobulin content. Acta Veterinaria Brno 2013;82:271–5.
[12] Miziolek M, Palleschi V, Schechter I. Laser-induced breakdown spectroscopy (LIBS) fundamentals and applications. Cambridge; 2006.
[13] Abdel-Salam Z, Abdel-Salam SAM, Harith MA. Application of laser spectrochemical analytical techniques to follow up spoilage of white meat in chicken. Food Anal Methods 2017;10:2365–72.
[14] Abdel-Salam Z, Harith MA. Laser spectrochemical characterization of semen. Talanta 2012;15:140–5.
[15] Kasem MA, Russo RE, Harith MA. Influence of biological degradation and environmental effects on the interpretation of archaeological bone samples with laser-induced breakdown spectroscopy. J Anal Atom Spectrom 2011;26:1733–9.
[16] Bilge G, Sezer B, Efe Eseller K, Berberoglu H, Topcu A, Hakki Boyaci I. Determination of whey adulteration in milk powder by using laser-induced breakdown spectroscopy. Food Chem 2016;212:183–8.
[17] Moncayo S, Manzoor S, Rosales JD, Anzano J, Caceres JO. Qualitative and quantitative analysis of milk for the detection of adulteration by Laser-Induced Breakdown Spectroscopy (LIBS). Food Chem 2017;232:322–8.
[18] Elnasharty YI, Kassem AK, Sabsabi M, Harith MA. Diagnosis of lubricating oil by evaluating cyanide and carbon molecular emission lines in laser-induced breakdown spectra. Spectrochim Acta B 2011;66:588–93.
[19] Abdel-Salam Z, Al Sharnoubi J, Harith MA. Qualitative evaluation of maternal milk and commercial infant formulas via LIBS. Talanta 2013;115:422–6.
[20] Khalil OM, Mingareev I, Bonhoff T, Richardson MC, Harith MA. Studying the effect of zeolite inclusion in aluminum alloy on measurement of its surface hardness using laser-induced breakdown spectroscopy technique. Opt Eng 2014;53:014106.
[21] Tegegne B, Tesfaye S. Bacteriological milk quality: possible hygienic factors and the role of Staphylococcus aureus in raw bovine milk in and around Gondar. Ethiopia Int J Food Contam 2017;4:1–9.
[22] Welearegay H, Yilma Z, Tekle-Giorgis Y. Hygienic practices and microbiological quality of raw milk produced under different farm size in Hawassa, southern Ethiopia. Agric Res Rev 2012;4:132–42.
[23] Armonk NY. IBM Corp. Released. IBM SPSS Statistics for Windows, Version 21.0.: IBM 484 Corp; 2012.
[24] Saito T, Yoshida T, Itoh T. Composition of free form glycopeptides isolated from bovine colostrum. Int Dairy J 1993;3:129–39.
[25] Roig MJ, Alegría A, Barbera R, Farré R, Lagarda MJ. Calcium dialysability as an estimation of bioavailability in human milk, cow milk and infant formulas. Food Chem 1999;64:403–9.
[26] Thapa BR. Health factors in colostrum. Indian J Pediatr 2005;72:579–81.

[27] Baker EN, Baker HM. A structural framework for understanding the multifunctional character of lactoferrin. Biochimie 2009;91:3–10.
[28] Anderson BF, Baker HM, Norris GE, Rice DW, Baker EN. Structure of human lactoferrin: crystallographic structure analysis and refinement at 2.8 A resolution. J Mol Biol 1989;209:711–34.
[29] Baker EN, Lindley PF. New perspectives on the structure and function of transferrins. J Inorg Biochem 1992;47:147–60.
[30] Carmona F, Muñoz-Robles V, Cuesta R, Gálvez N, Capdevila M, Maréchal J, et al. Monitoring lactoferrin iron levels by fluorescence resonance energy transfer: a combined chemical and computational study. JBIC 2014;19:439–47.
[31] Abdel-Salam Z, Abdel-Salam SAM, Abdel-Mageed II, Harith MA. Assessment of sheep colostrum via laser induced fluorescence and chemometrics. Small Ruminant Res 2017;155:51–6.