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Assessment of Milk Quality at Farm Level using Laser Techniques

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Abstract

Milk plays a vital role in different aspects along the chain. The profit of dairy farms is affected by many factors like milk production and quality. Mastitis disease is one of the key risks that lead to a decrease in the milk quantity and quality, particularly sub-clinical mastitis which attacked on dairy udders without visible signs. So, the aim of this study was to set a practical protocol to evaluate the condition of milk quality along the chain using laser techniques. Ninety-nine dairy cattle (Holstein and crossbred Holstein* Montbéliarde) at commercial farm in Egypt were used in this study. The foremilk samples were collected. Milk composition and somatic cell count (SCC) were evaluated. Forty samples were selected according to SCC to be tested by laser-induced breakdown spectroscopy (LIBS). The results obtained indicated that milk yield, protein %, solid not fat (SNF%), lactose% and density were decreased in the mastitis milk (subclinical and clinical) compared to normal milk. Also, there was a significant negative correlation between the Na (LIBS) score and milk content except fat %, and there was a positive correlation with SCC. This clear relationship could be proposed to assess the milk quality along the dairy chain using the LIBS technique to control quality, particularly at farm level.

Keywords: Milk quality, Na (LIBS) Score, Mastitis, SCC and Sub-clinical.

1. Introduction

Milk is one of the main food sources, according to its nutritional value which could lead to sustain life at different levels [1]. The profitability of dairy farms is significantly affected by milk production, nutritional value, and quality [2]. Ruegg [3] reported that the losses regarding to mastitis don't only concern to economic issues which were represented in reduction in milk yield and quality, excessive use of antibiotics or labor cost; but also, the losses are represented in the influence on dairy cattle welfare and health.

Mastitis is considered as the main disease that affect milk production and quality, where reduces the milk yield, and quality as well as increases the cost of treatments [4,5]. Furthermore, subclinical mastitis with higher risks that threaten the dairy sector compared with clinical mastitis. Subclinical mastitis could be led to a reduction in milk production by 10-20 % [6]. Also, challenges facing the dairyman regarding to subclinical mastitis are vital, where it's a silent or hidden disease, without any observations or abnormalities in milk or udder [7]. Where, the main changes in subclinical mastitis milk occur in physical and chemical composition. Therefore, a specific diagnostic tool is required to detect it as early as possible to control losses in the short and long term.

There are many changes in milk of sub-clinical mastitis such as changes in somatic cell count [2], mineral proportions in the milk, pH, electrical conductivity etc. Sodium (Na+), zinc (Zn) and chloride (Cl-) ions are increased in milk of sub-clinical mastitis quarters and consequently the electrical conductivity is higher compared to normal quarters [8,9]. Somatic cell counts, California mastitis test, and electrical conductivity of milk are a diagnostic test for detecting mastitis in cattle.

Recently, laser-induced breakdown spectroscopy (LIBS) has become commercially and widely used, particularly in the livestock sector, due to its fast, high accuracy and minimum or no preparation of samples [10,11]. Furthermore, the authors found that there was a positive relationship between Na (LIBS) and somatic cell count (SCC). Besides use of LIBS to assist sheep colostrum to control a nutritional approach of lambs

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[11]. Also, LIBS was conducted to detect milk adulteration [1]. The main objective of this study was to set a practical protocol to evaluate the condition of milk quality along the chain using laser techniques particularly at farm level.

2. Materials and methods

2.1. Study location:

Milk samples were collected from one commercial farm which located in Sharqia Governorate, Egypt. Ninety-nine dairy cows (Holstein and Holstein* Montbéliarde) were used to collect milk samples. Cows were in the middle of lactation period and parity from one to four.

2.2. Sampling and analyses of samples:

Three hundred milk samples were collected from ninety-nine dairy cows. Foremilk from each udder quarter was collected in Falcon tubes according to [12]. Milk samples were analysed by Lactoscan MCC Combo device (Bulgaria) at Faculty of Agriculture, Cairo University to measured milk composition (fat, protein, lactose, solid not fat (SNF), density), and SCC. Forty samples were selected according to SCC to be tested by LIBS technique.

Assessment of LIBS and multivariate analysis methods were used as tools to detect Mastitis. The applicability of LIBS connected with Chemometrics for identify healthy milk and Mastitis milk samples has been examined. LIBS spectra of normal and Mastitis milk samples were acquired through the spectral wavelength range of 200–900 nm. Data analysis was conducted through principal component analysis (PCA) and partial least squares (PLS) procedures. A score plot discriminating between healthy and mastitis samples, as well as their blind samples, was drawn utilizing principal component 1 (PC1) and principal component 2 (PC2).

2.3. Data analysis of LIBS:

LIBS spectra include a lot of data with high dimension that becomes complicated to manipulate with conventional statistical methods. Multivariate procedures support the exclusion of shot-to-shot laser fluctuations. Therefore, multivariate data analyses were conducted on the obtained LIBS data to differentiate between Mastitic and normal milk samples of a cow. PCA was the preferred method to reduce the size of the dataset while retaining as much information as possible. For this intent, the two principal components were applied to construct the PCA models. PLS is also a chemometric method that finds a linear regression model by transferring the X and Y variables into a new space. In the current study, modelling of infection rate as a function of LIBS spectra was estimated using the developed PLS calibration and models.

2.4. Statistical analysis:

General linear model (GLM) was applied by SAS® On Demand for Academics. Milk composition and SCC data were analyzed according to the follows model: $Y_{ijkmn}= \mu_i + Sj+d_k+L_m+e_{ijkmn;}$ where: Y_{ijkmn}= variable, μ= overall means, S_i= three SCC categories (where i= normal milk: <250000 SCC/ml; sub-clinical mastitis: 250000-٤٥0000 SCC/ml and clinical mastitis: ≥ ٤٥0000); d_j= days in milk as covariance; L_k= lactation period as covariance and e_{ijkmn}: standard error. The significant differences among groups were tested by Tukey procedure ($p<0.05$). Personal correlation between Na (LIBS) score and milk quality was applied Python (version 3.10.2).

3. Results and discussion

3.1. Milk composition and SCC

Figure (1) shows milk yield for normal milk (< 250000 SCC/ml) compared to sub-clinical (250000 to >450000 SCC/ml) and clinical mastitis milk (≥ 450000 SCC/ml). The milk yield was significantly higher in normal cows compared with sub-clinical and clinical cows. Also, milk quality in terms of protein%, SNF%, lactose% and density decreased as SCC increased (Table 1). Average days in milk and fat %, on the other hand didn't differ between categories ($P>0.05$).

In the dairy sector milk quantity and quality are very important from an economic point of view [13]. Milk production and quality are varying numerous factors such as genotype, management system, feeding system, seasons, lactation period as well as the health of udder [9,14]. Dairy animals are affected by mastitis disease, which has a serious impact on economic and animal welfare [15]. Moreover, this disease comes in two forms: clinical or subclinical that had a negative impact on udder tissues, milk yield and composition like protein, lactose, and fat [16]. These results were agreed with our findings in the current study (Figure 1 and Table 1), where mastitic milk (subclinical and clinical) had lower values in terms of milk production, protein, lactose, SNF, density than normal milk ($P<0.05$).

In Figure (2) shown the significant differences among different categories (normal, sub-clinical, and clinical) according to SCC, where the highest SCC observed in milk of clinical mastitis group then in sub-clinical milk group then normal milk group. There are many studies that have been addressed the threshold of SCC to distinguishing between normal milk or mastitis

milk (subclinical and clinical), where the SCC in normal milk does not exceed of 100000 [17,18], 150000 [19], 200000 [17,18,20], 250000 [21,22,23] or 300000 [10, 24] cells/ml. While the SCC in sub-clinical milk was exceed of 200000 [20], 250000 [21,23] 300000 cells/ml [19,24]. In the clinical mastitis milk, the SCC was greater than 400000 [25] or 500000 cells/ml [26]. However, lower levels of SCC may be considered for disease identification depending on the animals' health regulations in each geographical region.

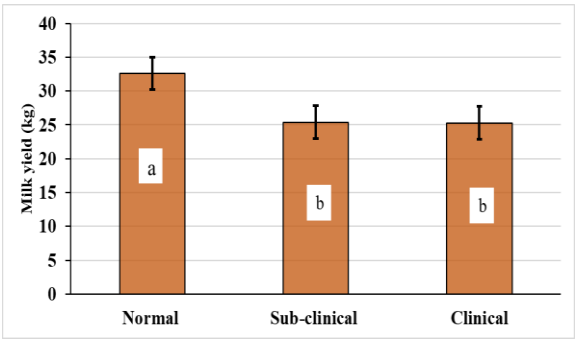

Figure 1. Milk yield of normal milk compared to sub-clinical and clinical Mastitis.
A statistically significant difference was shown by different letters (P < 0.05).

Table (1): Least square means ± SE of milk composition of three different milk status (normal, subclinical, and clinical mastitis milk).

Item	Normal	Subclinical	Clinical	P value
ADM	217±8.2	242±28.6	232±21.8	0.691
Fat %	2.1±0.1	2.4±0.2	2.4±0.1	0.081
SNF %	9.9±0.1^{a}	9.1±0.2^{b}	9.2±0.1^{b}	<0.0001
Protein %	3.6±0.02^{a}	3.4±0.07^{b}	3.4±0.05^{b}	<0.0001
Lactose %	5.5±0.03^{a}	5.0±0.1^{b}	5.0±0.08^{b}	<0.0001
Density	34.6±0.2^{a}	32.5±0.7^{b}	32.5±0.5^{b}	<0.0001

ADM: Average days in milk; SNF: Solid not fat. Different superscripts a and b at the same row are significant (P<0.05).

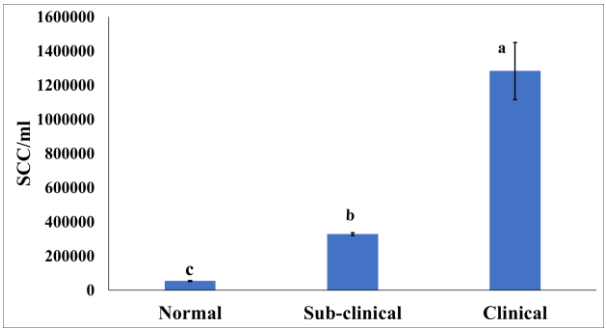

Figure 2. Somatic cell count (SCC) of normal milk compared to sub-clinical and clinical Mastitis.
A statistically significant difference was shown by different letters (P < 0.05).

3.2. LIBS analysis

Figure (3) depicts a typical LIBS spectrum of healthy and mastitic milk samples (upper). The given spectrum is the average of forty spectra gathered from ten various spots on the milk sample, as mentioned in the experimental part. Cyanide (CN) bands and Na spectral lines are mainly appearing due to two mechanisms.

In Figure (3) shown the mastitic milk with high SCC (>250×10^{3} cells/ml) had a high intensity of the CN band in the LIBS spectrum, which indicates its high protein level compared to the normal milk samples with low SCC (<250×10^{3} cells/ml). In addition, the mastitic milk samples were appeared with a high intensity of two Na lines in the same spectra in comparison with the normal milk, as illustrated in Figure (3). In the mammary glands, most of the milk proteins are synthesized [27]. Thence, the mastitis disease has a negative impact on the milk composition, in particularly essential milk protein and casein, as a result from the damage that happened in the mammary glands and the devastation of blood-born peptidases. Consequently, the contribution of casein to the rise of the Cyanide (CN) intensity may be ignored due to its decreased amounts with the increase of SCC and other proteins. The two Na D-lines intensities were highly observed in the mastitic milk compared to the healthy milk [10]. Sharma *et al.* [28], reported that with increases in SCC the sodium is increased as an impact of mastitis disease.

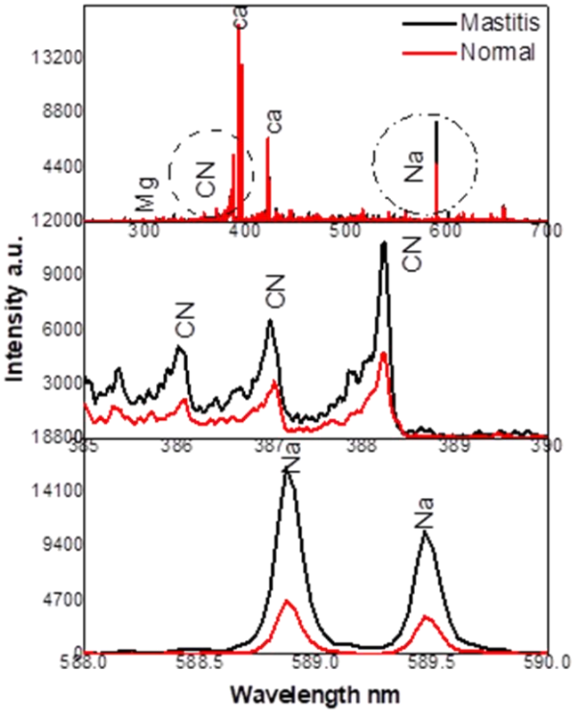

Figure 3. A typical LIBS spectrum for infected and normal milk samples.

3.3. PCA of the LIBS Spectra

In the current study, high dimensional LIBS data were assessed by chemometric procedure. The PCA multivariate statistical analysis method was utilized for distinction of normal and mastitic milk samples. PCA is widely employed in the cluster pattern and discrimination analysis in organic materials, notably in food science, animal health, etc. In the current study PCA analysis was executed for the LIBS spectra in the wavelength range of 200–900 nm. Three replicates were analysed for each sample, all replicates were used in the contribution of the PCA models. Hence, the samples were divided into two groups in the scores plot shown in Figure (4).

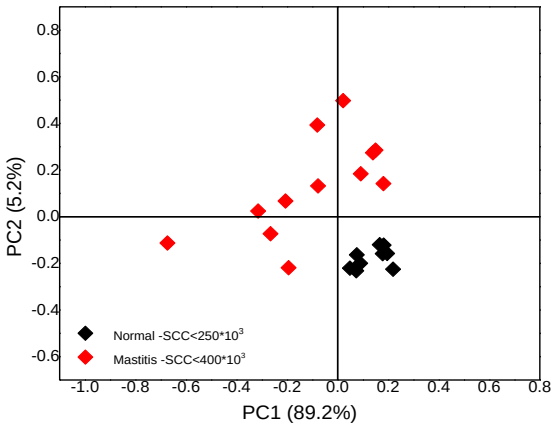

Figure 4. Principal component analysis (PCA) score plot for the LIBS spectra of the milk of a cow (normal and Mastitis).

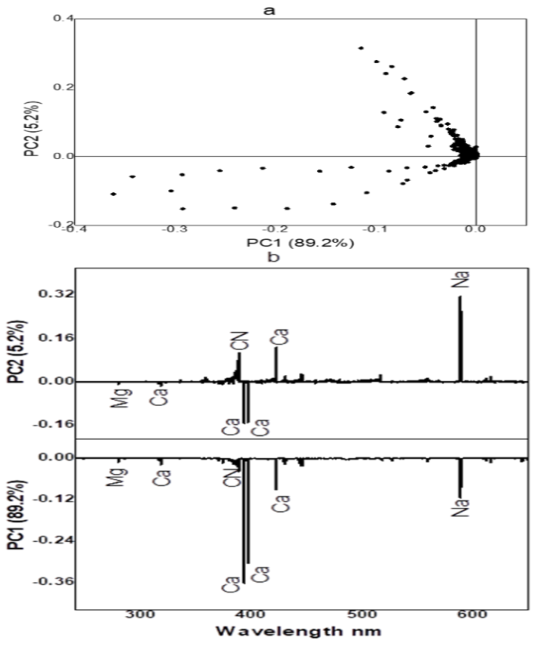

Figure 5. Principal component analysis (PCA) loadings plots PC1 vs. PC2. PC: principal component.

Though the PCA model was designed by using three principal components, the first two PCs were selected given their highest cumulative variance. The first PC held 89.2% of the variance, while the second PC held 5.2% of the variance. In Figure (4), clusters of healthy and mastitis samples were successfully achieved by using the developed PCA model. A comparatively highly clustering was noted in both groups (normal and mastitis milk).

Table (2): Band segment

Emission line (nm)	Elements
247.7	Iron
279.5	Magnesium
388. 2	Iron
393.3	Calcium
396.8	Calcium
386.0	**Cyanide**
388.0	**Cyanide**
422.6	Calcium
343.5	Calcium
345.5	Calcium
589.0	**Sodium**
589.6	**Sodium**

Figure (5) clarified the loadings plots of this principal component analysis. As the collected LIBS spectra in our study contain 28201 data points on the x-axis, it was not possible to show the exact spectral ranges on the loadings plot, which highly contributes to the discrimination success of the developed PCA model. Detailed analysis of Figure (5a) confirmed that the upper side of the plot was formed by the 588 to 591 nm spectral range, which is ascribed to the difference in Na content of the samples. Several spectral ranges have contributed to the lower side of the plot, which is attributed to differences in Fe, Ca, Mg, Na, and CN contents of the samples. The spectral ranges belong to two elements: mainly 386–388 nm (CN), and 589–589.6 nm (Na), as shown in Table (2). Similar results were obtained in Figure (5b), it can be seen that Na, Ca, and K content majorly contributed to the discrimination success, followed by differences in the Mg, Fe content. PC2 shows the significant contribution of Na and the minor contribution of CN content to PCA discrimination.

3.4. PLS analysis of the LIBS spectra

In the current research, the PLS has been used for quantitative analysis of the LIBS data obtained. Normal and mastitic milk sample LIBS spectra data were used to develop the calibration and validation models. The calibration graph of PLS is presented in Figure (6). A high coefficient of determination value was obtained for the calibration model (R^2=0.99).

Accordingly, to mastitis disease (subclinical and clinical) there is a change in the minerals of milk, for instance increasing the sodium (Na) and decreasing

the potassium (K) [29]. Nogalska *et al.* [9] reported that the relationship between milk contents and SCC have been widely studied. In our study there are a relationship among Na (LIBS) and milk yield and quality. Where the Na (LIBS) had a negative correlation with protein, SNF, lactose and density of milk (Figure 7). While Na (LIBS) and SCC were correlated positively, which agree with the finding of Abdel-Salam *et al.* [10] and Abdel-Salam *et al.* [30].

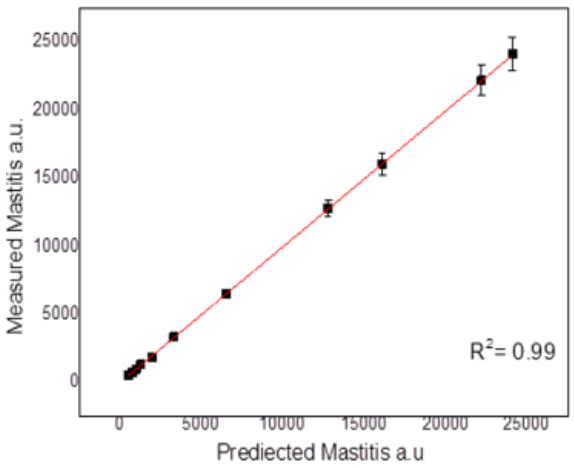

Figure 6. Partial least squares (PLS) calibration of mastitis milk of cow.

3.5. The correlation between Na (LIBS) score and milk quality

The correlation among milk production, milk quality and Na (LIBS) score were shown in Figure (7), where there was a positive correlation between Na (LIBS) score and SCC (P=0.0002). this result was agreed with Ogola *et al.* [31]. On the other hand, the Na (LIBS) score was correlated negatively with SNF, lactose, protein, and density (P≤0.005). Nogalska *et al.* [9] reported that the Na is higher in mastitic milk compared to normal milk which boost our observation.

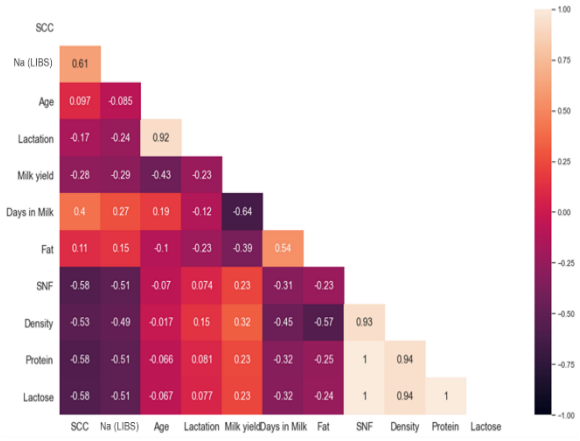

Figure 7. The correlation between milk production, SCC, milk composition and Na (LIBS) score.

4. Conclusion

Mastitis disease (subclinical and clinical) has a negative effect on milk production and quality. There is a negative relationship between milk component and Na (LIBS) score with highly significance. According to previous results LIBS could be used as a diagnostic tool to assess the milk quality as well as SCC through the dairy chain and particularly at farm level.

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6. Conflict of interest: The authors state that there is no conflict of interest.

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