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Application of Laser Spectrochemical Analytical Techniques to Follow Up Spoilage of White Meat in Chicken

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Abstract The overall objective of this paper is to evaluate the potential of laser spectrochemical analytical techniques as rapid, cost-effective, and accurate techniques to detect the onset of spoilage in fresh chicken breast fillets in three consecutive days directly following slaughter day. Samples were periodically examined via laser-induced breakdown spectroscopy (LIBS) and laser-induced fluorescence (LIF). In the case of LIBS, the cyanide (CN) and carbon (C_2) molecular spectral emission bands in the LIBS spectra of meat have been taken as indicators of protein content in the chicken breast samples. The ratio of ionic to atomic spectral lines of both magnesium and iron is found to be proportional to the chicken meat tenderness which decreases with storage time. LIF has been also exploited as a simple and fast technique for white meat spoilage detection. There was a clear inverse proportionality between the intensity of the samples' fluorescence band and the storage period. The obtained spectrochemical results have been validated by measuring the total proteins in the investigated samples using a conventional meat analyzer. This work demonstrates the feasibility of adopting LIBS and LIF techniques in characterization of both fresh and spoiled chicken meat samples.

Keywords Spectrochemical analysis · LIBS · LIF · White meat · Meat spoilage

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Introduction

Meat is an essential component in the human diet and represents the essential source of proteins among other foods. Therefore, new techniques for meat conservation, refrigeration, quality control, and analysis have been developed to guarantee its high quality. One of the most highly perishable foods is chicken meat; therefore, it is very important to develop methods for estimation of its spoilage in order to monitor the quality of such type of meat (Guevara-Franco et al. 2010; Lin et al. 2004; Sahar et al. 2011). In fact, there are many factors that affect meat spoilage, such as poultry health, age, and sex, as well as condition of chicken carcasses at slaughter time, packaging type, and storage conditions (Huis in't Veld 1996). Raw chicken meat may deteriorate in relatively short time of about 4 to 10 days, even when stored at low temperature (Lin et al. 2004; Sahar et al. 2011; Jiménez et al. 1997).

Nowadays, the consumers' demand for chicken meat is increasing, due in part to its reasonable cost and also for dietary health considerations (Sahar et al. 2011; Ellis et al. 2002). The distributors as well as the consumers require long expiry times for meat of guaranteed good quality. This demonstrates the challenges that face the meat industry in order to improve the handling and processing procedures to elongate the meat shelf life under optimum conditions (Nychas et al. 2008; Balamatsia et al. 2006). Sensory evaluation is the most frequently used for freshness assessment of meat and meat products in general. However, there are other approaches used for this task adopting microbial count methods, such as volatile compound analysis, total viable counts, and total volatile basic nitrogen, biogenic amine index, and measurement of lipid oxidation (Balamatsia et al. 2006; Balamatsia et al. 2007). All these methods are costly, lengthy, and technically complicated. Consequently, it is urgent to develop new rapid and cost-effective

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techniques to detect and monitor meat spoilage for continuous guarantee of its high quality (Lin et al. 2004; Sahar et al. 2011; Ellis et al. 2002; Guevara-Franco et al. 2010).

Laser spectrochemical analytical techniques, namely laser-induced breakdown spectroscopy (LIBS) and laser-induced fluorescence (LIF), have been shown to provide the most sensitive detection of the elements and molecules, respectively (Noll 2012; Andresen 2001). In fact, LIBS is a powerful elemental analysis technique that can be used to analyze numerous types of samples including the biological ones (Abdel-Salam et al. 2006; Abdel-Salam and Harith 2012; Hassan et al. 2008; Kasem et al. 2011; El-Hussein et al. 2010).

This technique has been developed for rapid measurement of the elemental composition of the samples and is well suited for production control monitoring (Elnasharty et al. 2011). The most attractive advantages of LIBS are little or no sample preparation is required, minimally destructive, enables direct measurement from a distance, and can be used for in situ measurements. LIF is a highly sensitive spectrochemical analytical technique widely used in studying the molecular structure. LIF requires no or minimal sample preparation, and it is also completely nondestructive and noninvasive, as well as being a rapid and time-saving technique; hence, it is the most appropriate for biological sample analysis. In situ and field applications of LIF are available due to its compact and simple equipment which facilitate its use in animal production farms for diagnosis of animals' diseases (Abdel-Salam et al. 2015), sperm count (Abdel-Salam and Harith 2012), evaluation of colostrum in milk (Abdel-Salam et al. 2014), etc.

Within food research, LIBS and LIF are still in its infancy. The LIBS technique has been applied for detecting adulterated meat (By Editor 2016) and for monitoring of Ca content in poultry meat (Andersen et al. 2016). To the best of our knowledge, no prior studies on using LIBS and/or LIF for detection or follow-up of meat spoilage have been reported.

This work demonstrates the feasibility of using LIBS and LIF as rapid, technically simple, and relatively cheap techniques to follow up in time spoilage of commercially available chicken white meat.

Material and Methods

Collection of Chicken Meat Samples

A total of 30 fresh samples from chicken breast fillets (each sample weighs approximately 200–300 g without skin) were collected from distinguished local supermarkets in the vicinity of our laboratory in Cairo University. These samples were transported to the laboratory within 30 min. All 30 samples were analyzed via LIBS and LIF techniques immediately, and this first analysis is considered as the starting point of storage (the first day after slaughter day). Samples were stored at 5 °C and analyzed again on the second and third day of storage. It is worth mentioning that in local supermarkets, meat should be frozen the fifth day after slaughter.

LIBS Arrangement

LIBS is a well-known spectrochemical analytical technique for elemental analysis that has been exploited in numerous biological applications during the last two decades. In LIBS, focused Q-switched nanosecond laser pulses produce the so-called plasma plume onto the surface of the target to be analyzed. The plasma plume consists of a collection of ions and swirling electrons at enormously high temperature (>6000 K). The light emitted from the cooling down plasma plume is collected by a proper lens system or optical fiber and fed to a suitable spectrometer equipped with a detector for dispersion and detection of the plasma emission light. Collection of the emitted light should be delayed for a certain period of time, called the delay time (in the microsecond range), with respect to the plasma onset to avoid the overwhelming bright emission in the early time of the plasma evolution. The integration time or the gate width is then the time interval during which the detector is sensitive (Noll 2012). The finally obtained spectrum includes the characteristic spectral lines of the elements existing in the plasma plume and consequently in the target material. Detailed description of the LIBS technique is found in a number of books and review papers (Fortes et al. 2012; Baudelet et al. 2007; Lucena et al. 2011; Dave and Ghaly 2011). In the present work, we used a typical LIBS setup in which a Q-switched Nd:YAG laser (Brilliant EaZy, Quantel, France) produces laser pulses of 100 mJ/pulse at a wavelength of $\lambda = 1064$ nm and pulse width of 5 ns at a repetition rate of 1 Hz. The laser pulses were focused in air under atmospheric pressure onto the surface of the sample under investigation using a planoconvex quartz lens of focal length 10 cm. The sample was fixed on an X-Y micrometric translational stage. The light emitted from the laser-produced plasma plume was collected via a 2-m-length and 600-μm-diameter fused silica optical fiber and fed to an echelle spectrometer (Mechelle 7500, Multichannel, Sweden) equipped with an ICCD camera as a detector (DiCAM-Pro, PCO, Computer Optics, Germany).

Spectra were collected from 10 laser shots on each of 5 fresh spots separated by 5 mm on the same meat sample to compensate for any inhomogeneity in the sample (a total of 50 spectra were collected for each sample). Spectra processing and spectral line identification have been performed by the commercial software LIBS++. Detailed description of the instrumentation is given elsewhere (Abdel-Salam et al. 2015).

LIF Arrangement

In LIF, laser light excites atoms or molecules which send out radiation when they undergo de-excitation. In the time interval between the excitation and de-excitation, nonradiative processes can occur that may have the effect of new state population. Since many different states are populated, fluorescence can take place on several wavelengths and it is possible to observe an emission spectrum. Because both the wavelength that excites the molecule and the one that is observed could be chosen, this makes an excellent choice to study atoms and small molecules. In an excited molecule, many processes can take place. An atom or a molecule can be excited in one way, by absorbing a photon, but it can be de-excited in several ways. It can spontaneously emit a photon, it can be stimulated to emit a photon, it can be quenched, or it can be photo-ionized and pre-dissociated. The excited species will de-excite after some time, usually in the order of few nanoseconds to microseconds, and emit light at a characteristic wavelength longer than the excitation wavelength. Such de-excitation emitted light can be measured for identification of the species under investigation and for other different tasks.

The experimental setup of the LIF equipment used throughout the present work was described in details elsewhere (Abdel-Salam et al. 2015). The laser used was a CW-DPSS laser [Changchun New Industries Optoelectronics Tech. Co., Ltd. (China)] of 5 mW power at $\lambda = 266$ nm. The laser beam was directed to one side of the chicken meat sample via a special annular optical fiber that delivers the laser beam and collects the fluorescent light. Laser delivery and fluorescence collection were performed perpendicular to the target surface. Each fluorescence spectrum is the average of 10 spectra collected from 10 different spots randomly distributed on the surface of the same sample to avoid any inhomogeneity. The fluorescence light was then fed to the entrance slit of the spectrometer (USB2000 FLG, Ocean Optics, FL, USA). This spectrometer has grating with 600 lines/mm, set to 350–1000 nm (blazed at 500 nm). The fluorescent spectrum is recorded in the spectral wavelength range from 375 up to 800 nm. Acquisition and further processing and analysis of the obtained spectra were accomplished using the commercial SpectraSuit software (Ocean Optics, FL, USA). The final processing of the data for presentation was performed with Origin 9.0 software (Origin Lab. Corp., MA, USA).

Laboratory Determination of Total Protein

Total protein has been measured in the chicken meat samples using classical meat analyzer (FoodScan ™ Pro Meat Analyzer, Foss Analytical A/S, Model 78810, Denmark) at the central laboratory of the Faculty of Agriculture, Cairo University. The automated system gave the values of total protein in percent, and the average for each group of samples representing certain storage time has been considered.

Statistical Analysis

Collected data were analyzed using IBM SPSS Statistics 21 (Armonk 2012). Descriptive statistical procedure was used to estimate means and standard deviation of different studied variables. The Duncan procedures were used to test the significant differences between the least square means of storage days and sources of samples. Statistical data analysis of the results was performed using analysis of variance (ANOVA) and Duncan test, at a 5% level of significance ($P < 0.05$). "P (sig.)" <0.05 indicates a statistically significant difference between two means, and P stands for probability. ANOVA was carried out to check the existence of statistically significant effect on the days of storage of samples.

Results and Discussion

LIBS Analysis

As mentioned above, meat is a major source of protein in human diet. Human and animals share the same components of their muscles; therefore, diet containing animal's tissue (meat) is a direct way to make use of its protein content. Proteins consist mainly of amino acids which represent its major structural and functional components. Because of the fact that LIBS is an elemental analysis technique, it has been used in many researches to detect and monitor some molecules such as CN, C_2, and OH through the presence of their molecular emission bands in LIBS spectra (Kasem et al. 2011; Elnasharty et al. 2011; Fortes et al. 2012). Figure 1 depicts a typical LIBS spectrum of breast chicken meat. The presented spectrum is the average of 50 spectra collected from five different spots on the sample as described in the experimental section. The appearance of CN and C_2 spectral bands is mainly due to two mechanisms. The first is recombination of molecular carbon with nitrogen coming from the target material or from air and recombination of atomic and ionic carbon with atomic or molecular nitrogen to form CN, while fragmentation is essential for C_2 production in molecules containing carbon-carbon double bonds (Baudelet et al. 2007; Lucena et al. 2011). The second mechanism is the direct ablation of native CN and C_2 molecules from the sample materials (Kasem et al. 2011). This shows that it is possible to make use of LIBS to evaluate proteins in meat samples through CN and C_2 spectral bands of both fresh and spoiled meat samples.

As shown in Fig. 2 (upper), the high intensity of the CN band in the LIBS spectrum of the fresh meat (first day) reflects its high protein content compared to the samples in the following 2 days. Similarly, the intensities of the C_2 spectral band

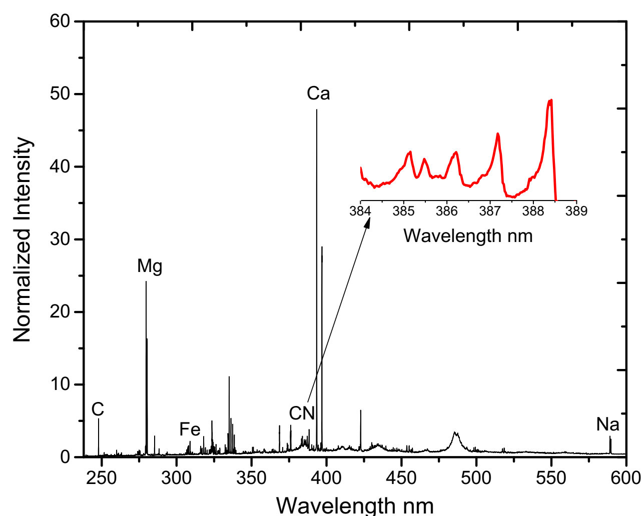


Fig. 1 Typical LIBS spectrum of breast chicken meat. The *inset* is the zoomed CN band

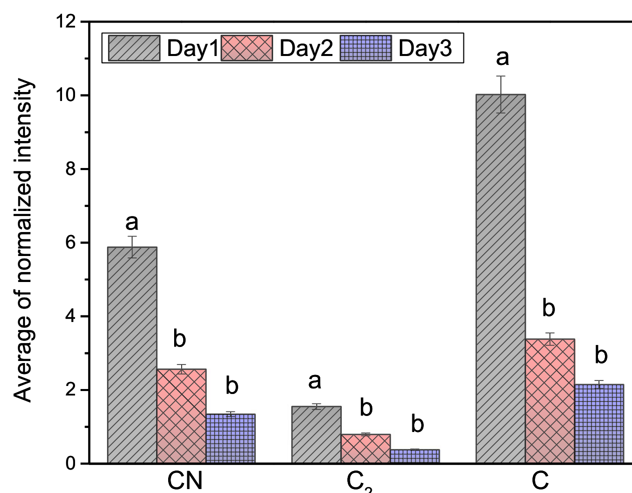
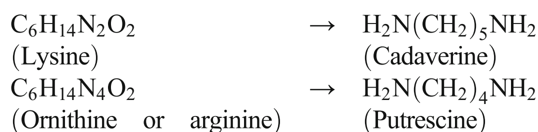


Fig. 3 Bar graph of CN, C_2, and C average normalized intensities at different storage times (calculated for the 30 breast chicken meat samples and from the 10 laser shots on each of 5 fresh spots). The *error bars* represent the standard deviation of the experimental data. *Different letters* (*a*, *b*) indicate significant differences ($P < 0.05$)

and the strong atomic line of carbon at 247.8 nm shown in Fig. 2 middle and lower, respectively, confirm the same results. In fact, LIBS results cannot discriminate between different types of protein, but it can be useful in evaluating the total protein in different meat samples. Figure 3 demonstrates the standard deviation of 1500 spectra (30 samples × 10 laser shots × 5 fresh spots) for CN, C_2, and C for the three storage days. The figure shows clearly the statistically significant differences between the first, second, and third storage days.

Protein degradation and loss of other valuable molecules are the consequence of meat spoilage process; this has been demonstrated also by (Dave and Ghaly 2011). Breakdown of proteins and lipids produces new compounds which change meat flavor, tenderness, juiciness, odor, and texture. Diamines, cadaverine, and putrescine are among the main metabolic by-product indicators of meat spoilage (Jay et al. 2005). The production of these diamines occurs in the following manner:



$$C_6H_{14}N_2O_2 \text{ (Lysine)} \rightarrow H_2N(CH_2)_5NH_2 \text{ (Cadaverine)}$$

$$C_6H_{14}N_4O_2 \text{ (Ornithine or arginine)} \rightarrow H_2N(CH_2)_4NH_2 \text{ (Putrescine)}$$

The two equations indicate that there is a loss in the carbon content due to the degradation processes. This lost carbon could be used by the bacteria growing on the sample surface as will be explained later. Hence, it is expected to have lower

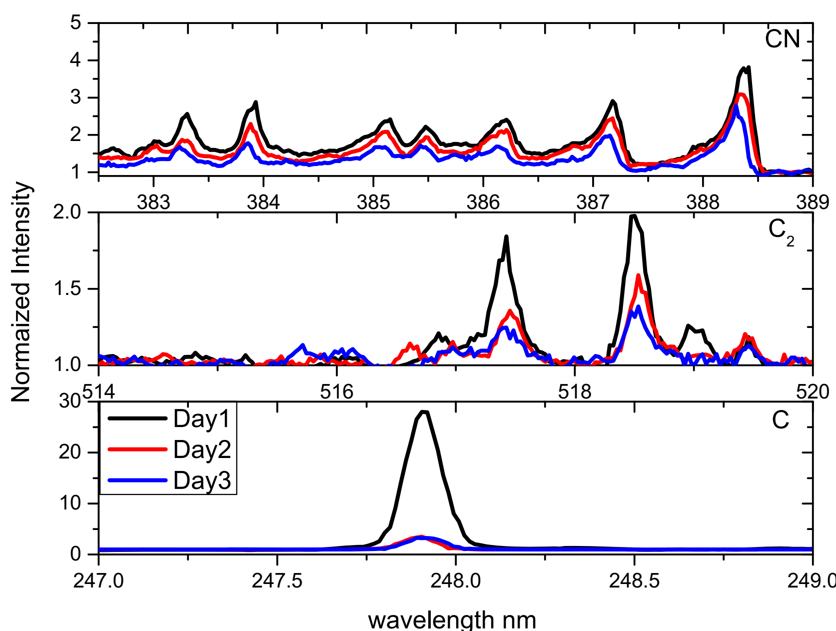


Fig. 2 Comparison between the violet CN band (*upper*), the Swan C_2 band (*middle*), and the C 247.8-nm atomic line (*lower*) in breast chicken meat at three different storage times in typical LIBS spectra

CN and C_2 signals in the LIBS spectra of spoiled meat samples.

The time evolution of the CN band around 388.3 nm is shown in Fig. 4. Ten spectra at five different spots of the meat sample have been taken for each delay time to estimate the mean line intensity and the standard deviation in each case. The trend of the obtained temporal behavior in which the intensity of CN band decreases exponentially with increasing delay time indicates contribution of native CN vaporized from the meat sample (Kasem et al. 2011).

Surface hardness of solid targets which corresponds to tenderness in meat is one of the main factors deciding the freshness of the meat. Samples having hardness of circa 450–500 N can be considered tender enough to fulfill the meat quality conditions (Chambers and Bowrs 1993). Hardness can be estimated via the ratio of ionic to atomic spectral line intensities in LIBS spectra (Abdel-Salam et al. 2007). The magnesium and iron spectral lines in the LIBS spectra of meat samples have been used to estimate the samples' hardness. The bar graphs in Fig. 5 show the values of the ratio Mg(II) 280.26/Mg(I) 285.22 nm and Fe(II) 285.67/Fe(I) 373.71 nm. The intensities of the lines are taken from the calculated mean values of the 1500 spectra (30 samples × 10 laser shots × 5 fresh spots). The figure depicts that the intensity ratio decreases with storage time, which means that the samples lose its tenderness with time. The main loss (68%) takes place after the first 24 h of storage in good agreement with previous work (Karlovic et al. 2009). These results confirm the above findings concerning the samples' degradation. It is worth to mention that Soltanizadeh et al. (2008) observed that the texture characteristics for different meats are influenced by postmortem proteolysis of myofibrilar proteins.

In the present study, spoilage of meat after 24 and 72 h from storage causes loss of tenderness in comparison to fresh meat due to loss of the organic material due to the breakdown of proteins. This, of course provides a favorable medium for bacterial growth (Miller 2002).

LIF Analysis

Laser-induced fluorescence is an important sensitive spectrochemical analytical technique that has been used in the present work for the identification of meat spoilage. Figure 6 shows the fluorescence spectra of the average of 300 spectra (30 samples × 10 spectra) of meat samples after three consecutive storage days. It is clear that there are two distinguishable fluorescent peaks in such spectra. The first which is around 492-nm wavelength decreases with storage time, while the second peak is at 696.08 nm and has an increasing intensity with storage time.

The intensity of the first fluorescent spectral peak is a function of the total protein level in the measured samples. The decrease in the fluorescence intensity in this case is due to the reduction of tryptophan from meat which is higher than that originating from the microorganisms (Oto et al. 2013).

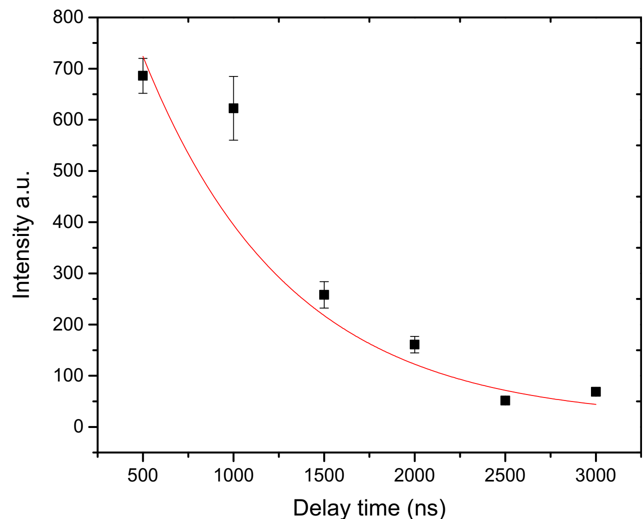


Fig. 4 Time evolution of the CN band around 388.3 nm. The *error bars* represent the standard deviation of the data

Figure 7 depicts a comparison of fluorescence band intensities of meat at 492 nm for mean of 300 spectra at different storage times. The intensity of the LIF signal of the first storage day is significantly high (P 0.05) with respect to the second and third storage day intensity values. In order to validate the spectrochemically obtained results, the total protein content in the samples has been measured via a conventional meat analyzer.

Figure 8 shows the decrease of the protein contents with storage time in agreement with the results obtained from both the LIBS and LIF measurements. It is well known that meat is able to spoil relatively fast, since it possesses an ideal

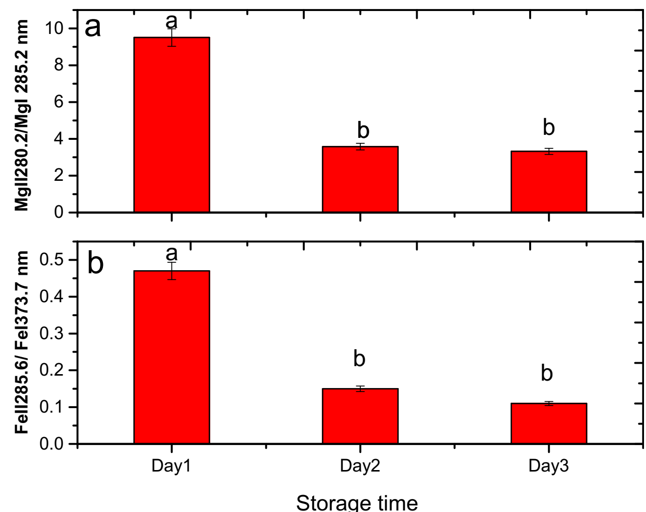


Fig. 5 LIBS intensity ratios of ionic to atomic spectral lines of magnesium (**a**) and iron (**b**) in chicken breast meat at different storage times. The *error bars* represent the standard deviation of the experimental data of each group. *Different letters* (*a*, *b*) indicate significant differences ($P < 0.05$)

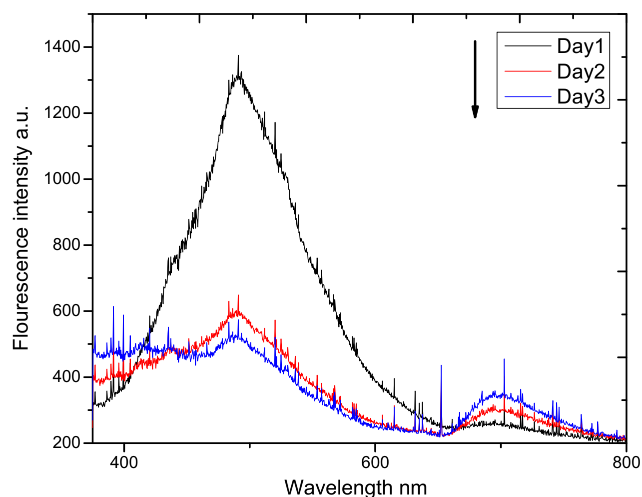


Fig. 6 The average of typical fluorescence spectra of chicken meat (30 samples × 10 spectra) at different storage times

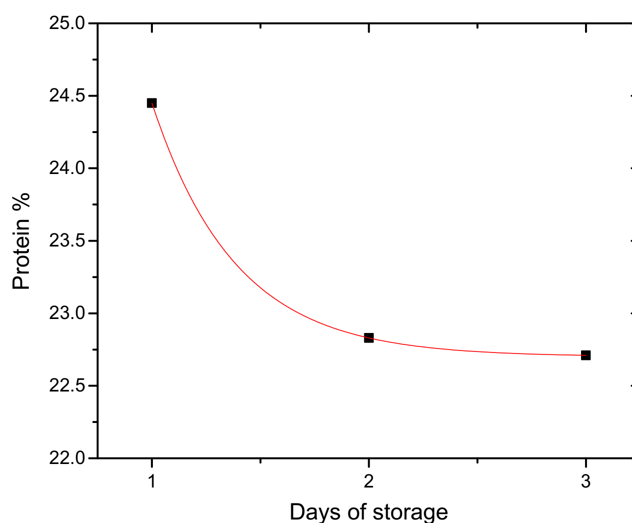


Fig. 8 The percentage changes of the protein content in chicken meat for the three studied storage days

composition for the growth of numerous microorganisms. Changes in color, odor, texture, taste, etc. are relevant to the spoilage of meat. Spoilage organisms primarily belong to the genus pseudomonas, as they attach more rapidly to meat surface compared to different spoilage microorganisms. The carbon needed as nutrient for bacterial growth is provided by the degradation of proteins as has been demonstrated by the equations shown above. Contrary to the first peak at 492 nm, the second peak intensity at 696.08 nm increases along with the spoilage time as shown in Fig. 6. In order to confirm the above-obtained results and explain the presence of the second fluorescence peak at 696.08 nm, a calibration curve between

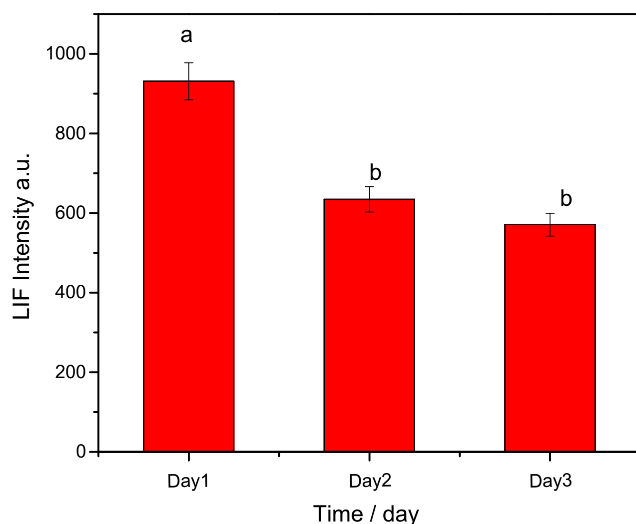


Fig. 7 Trends of average integrated fluorescence intensity values of 30 chicken meat samples at different storage times. The *error bars* represent the standard deviation of the data. *Different letters* (*a*, *b*) indicate significant differences ($P < 0.05$)

the fluorescence intensity as a function of the colony-forming unit (CFU) has been constructed adopting different concentrations of *Pseudomonas aeruginosa* (DSM50090). Samples of bacteria having concentrations from $\log 10^7$ to $\log 10^2$ CFU/cm^2 have been used. *P. aeruginosa*: Bacterial cultures were seeded and incubated at 37 °C for 24 h. After this period, all strains were stored in 0.95% normal saline with 50% glycerol 1:1 at −20 °C. Growth media used for bacterial growth were Trypticase soy broth and Trypticase soy agar (OXOID®, Hampshire, England). All dilutions were made in sterile 0.9% normal saline (Sousa et al. 2015). The fluorescence intensity was then measured for the band at 696.08 nm for different bacterial concentrations measured in CFU/cm^2.

The plot of the fluorescence intensity versus the concentration CFU/cm^2 of *P. aeruginosa* is shown in the inset in Fig. 9. The results obtained depict a linear relationship between the fluorescence intensity response and the *P. aeruginosa* over the entire investigated range of bacteria concentration. The slope of the calibration curve at a certain concentration is termed the sensitivity, and it gives the change in the signal (fluorescence intensity) for a given incremental change in the *P. aeruginosa* concentration. The regression factor (R value) is given on the calibration curve in Fig. 9. The fluorescence peak at 696.08 nm of the meat samples corresponds to the increase in the bacterial spoilage. Such linear relationship (R = 96) demonstrates the feasibility of using LIF in obtaining the spoilage stage of any meat sample adopting the relevant calibration curve. Sahar and Dufour (2014) found that in chicken breast meat stored at 5 °C, pseudomonas grew significantly from day 0 to day 8 and became the most abundant microorganisms on day 3 (7.4 log CFU/cm^2), which is in good agreement with the results obtained in the present work.

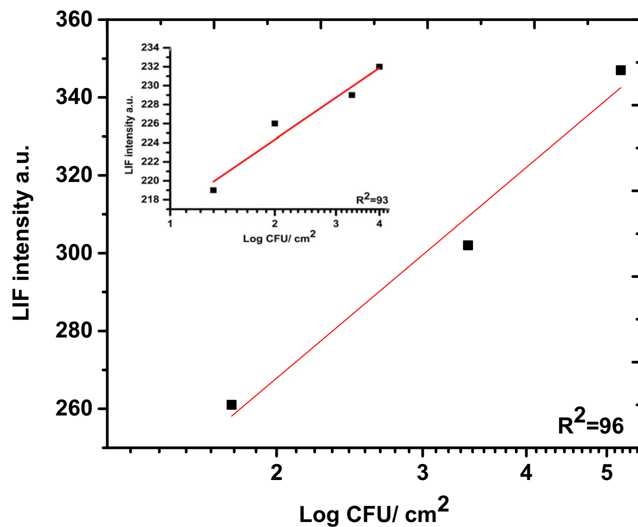


Fig. 9 Calibration curve for fluorescence intensity values of meat versus the log concentration CFU/cm^2. The *inset* depicts the calibration curve for fluorescence intensity values of *Pseudomonas aeruginosa*

Conclusion

In conclusion, results reported in the present paper show that the use of laser spectrochemical analytical techniques provides new and fascinating possibilities for detecting the onset of spoilage in fresh chicken breast fillets in three consecutive days directly following slaughter day. In the present study, proteins have been evaluated using the spectral lines of the molecular bands of CN and C_2 in the LIBS spectra. The ratio of Mg(II)/Mg(I) and Fe(II)/Fe(I) is found to be proportional to the chicken meat tenderness which decreases with storage time. In addition, it has been demonstrated that with LIF, it was possible to detect spoilage of chicken meat after different times of storage. The potentials of LIF have been demonstrated in relating the sample protein content and the relevant laser-induced spectral band fluorescence intensity. The results obtained via LIBS and LIF techniques concerning the protein degradation with storage time have been validated by analyzing the meat samples with a conventional meat analyzer.

Compared with other previously published works in this field, this work presents successful spectrochemical laser techniques, namely LIBS and LIF as cost-effective, noninvasive, nondestructive, and fast analytical methods that can be exploited to follow up spoilage of chicken meat.

Compliance with Ethical Standards

Conflict of Interest Z. Abdel-Salam declares that she has no conflict of interest. S.A.M. Abdel-Salam declares that he has no conflict of interest. M.A. Harith declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

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