

## Applicability of Biosensor and Oxygen Sensor for Monitoring Spoilage and Bacterial Contaminants of Packed Minced Beef and Poultry

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### Abstract

New technologies have revolutionized the food safety sector. Nano-biosensor based enzyme and oxygen sensor have recently gained much attention in the field of food safety. Two prototypes of the sensor capable of handling of different samples were developed and tested with beef and chicken fillets that stored at 4 °C±1 for 11 days, as well as the physicochemical, freshness and microbiological tests were done. The results from this study demonstrated that there are strong color changes were observed in biosensor array from a green color to red color after 9 and 10 days of storage beef and chicken fillets, respectively. Likewise, increased in pH, TVB-N and TBA values during the storage periods. Moreover, the TVBC and psychrophilic bacteria were increased. Also, noticed that the remaining oxygen content in the package headspace of different samples decreased continuously during storage. These data present promising evidence for possible application of bio- and oxygen sensor for rapid detection of meat spoilage for food defense and food safety application.

**Keywords:** nanotechnology, biosensor, oxygen sensor, beef, chicken fillets, microbiological quality.

### Introduction

Nanotechnology has emerged as one of the most innovative technologies and has the potential to improve food safety and quality. Safe food is an essential requirement of modern society, and governmental authorities throughout the world are constantly monitoring the food supply chain in an attempt to ensure an adequate safety level (Neethirajan and Jayas, 2011). Likewise, this concern for food safety has recently been further exacerbated by the growing threat of bioterrorism (Ko *et al.*, 2010).

According to published CDC 2011 (US Centers for Disease Control and Prevention) data, foodborne diseases account for approximately 76 million illnesses, 325,000 hospitalizations and 5000 deaths each year in the US alone. While meat and poultry have been implicated in several foodborne outbreaks over the years, these products also are prone to spoilage (Cardenas *et al.*, 2008).

Many methods have been employed to detect pathogens and microbial contaminant; however, some methods, such as traditional colony counting, are excessively time consuming while others, PCR for example, involve complex instrumentation and handling (Lazcka *et al.*, 2007). Moreover, determination of the TVB-N is one of the most widely used methods to measure the freshness of meats. The total volatile base measured is composed principally of ammonia, trimethylamine (TMA) and dimethylamine (DMA) (Ocano-Higuera *et al.*, 2009). All these methods are relatively time consuming, require experienced personal and expensive apparatus. The demand of novel technologies for the rapid detection of food spoilage and bacterial

contamination becoming an increasingly urgent (Scindia *et al.*, 2007).

Biosensors are compact analytical devices which detect, record and transmit information pertaining to biological reactions (Yam *et al.*, 2005). They consist of a bio-receptor specific to a target analyte and a transducer to convert biological signals to a quantifiable electrical response. Bio-receptors are organic materials such as enzymes, antigens, microbes, hormones and nucleic acids. Transducers may be electrochemical, optical, or calorimetric and are system dependent (Zhang *et al.*, 2014). Intelligent packaging systems incorporating biosensors have the potential for extreme specificity and reliability. A market analysis of pathogen detection and safety systems for the food packaging industry suggests that businesses offer considerable promise for future growth (Alocilja and Radke, 2003). Biosensor methods can be applied, as rapid screening tools, to detect such contaminants. They can be developed using several types of transducer (Reder-Christ and Bendas, 2011) one of which is based on fluorescence either through quenching (Wang *et al.*, 2011) or labeling of the biological recognition elements, e.g., fluorescently labeled antibody (Taitt *et al.*, 2008). The market for biosensors was estimated as \$17.3 billion by year 2015 as per some business reports (Sadana and Sadana, 2011).

Fluorescence-based oxygen sensors represent the most promising systems to date for remote measurement of headspace gases in packaged meat products. A number of disposable oxygen sensing prototypes have been developed which may be produced at low cost and provide rapid determination of oxygen concentration (Kerry and Papkovsky, 2002). The active component of a fluorescence-based

oxygen sensor usually consists of a long-delay fluorescent or phosphorescent dye encapsulated in a solid polymer matrix (Borchert *et al.*, 2012).

The aim of the present research was to develop, from first principles, a low cost, potentially portable, optic biosensor capable of detecting low levels of microbial contaminants and compounds of interest in food analysis. Also, attempt to use non-invasive oxygen determination method based on fluorescence quenching to evaluate the shelf-life of meat.

## Materials and Methods

### Sample collection and preparation

Minced beef and chicken fillet samples used in this study were purchased from a local market in Lyngby City, Denmark (DK). The samples were transported in the isothermal ice box to the laboratory within 10 min. The samples were cut into portions 5 cm x 5 cm; this procedure was performed in a hood cabinet with an aseptic knife. All prepared samples were put in a sealed plastic bag, and stored at  $4 \pm 1^\circ\text{C}$ .

### Design of biosensor array

Biosensor array is an enzymatic system, was based on color change caused by a pH decrease which is the result of a controlled enzymatic hydrolysis of a lipid substrate. Hydrolysis of the substrate cause acid release and the pH drop is translated in a color change of a pH indicator from deep green to bright yellow to orange red. Different combinations of enzyme-substrate types and concentrations can be used to give a variety of response lives, temperature dependencies and spoilage stage. Biosensor is inactive until pressure is applied to break a barrier between two "ampoules" and the contents are mixed together. One ampoule contains a proprietary lipase enzyme and pH indicating dye while the other ampoule contains an enzyme substrate (triglyceride). A green to yellow to orange red color change occurs as the pH is lowered via liberation of fatty acids from triglyceride by the lipase enzyme. Since excess enzyme is present, the reaction rate is governed primarily by temperature. Concentrations of lipase enzyme and triglyceride can be manipulated during manufacture to yield targeted activation energies,  $E_a$ , providing design flexibility (Vitsab A.B., Malmö, Sweden).

### Oxygen sensor technique

The oxygen in the jar's headspace of meat samples was monitored, using an optical measuring system Optech™ Platinum O<sub>2</sub> sensor device and disposable O<sub>2</sub> sensor stickers from Mocon (Minneapolis, USA). The optical O<sub>2</sub> sensor works on the principle of a phosphorescent dye that is incorporated in a polystyrene polymer membrane as adherent agent and for environmental protection. The Optech device uses LED technology to take

measurement in 10 s. For O<sub>2</sub> measurements, the instrument need to be brought in an optical contact with the sensor (5–10 mm distance) to produce an O<sub>2</sub> reading (% of O<sub>2</sub>, compensated for temperature and pressure variation). The OxyDot-indicators were placed in the headspace of the sample jars using transparent adhesive. A Windows-based software was used for the recording of measured parameters (oxygen concentration) and data storage (Molinario *et al.*, 2013).

### Physicochemical analysis

Moisture content, crude protein, crude fat and total ash were determined according to A.O.A.C. (2005). The pH measurements were carried out using a digital pH-meter (model MA 5736, Metrel, Iskra, Slovenia). The contents of TVB-N and TMA were determined by steam distillation according to the method described by (Harold *et al.*, 1987) and expressed as mg N/100g of muscle. TBA was determined using a spectrophotometric method according to (Vyncke, 1970) and the results were expressed as mg malondialdehyde (MDA)/kg of muscles.

### Microbiological Analysis

A sample of 25 g was taken aseptically from each fillets and transferred to a stomacher bag, and 225 mL of sterilized peptone water (Becton, Dickinson and Co., Le Pont de Claix, France) was added. The mixture was homogenized for 2 min with a Stomacher 3500 (Seward Medical, Worthing, U.K). Samples (0.1 mL) of serial dilutions of fillet homogenates were spread on the surface of the appropriate dry medium in Petri dishes for determination of the total viable bacterial count (TVBC) and psychrophilic bacteria (Psy.) on plate count agar (Oxoid, CM325), and incubated at 37 °C for 48 hrs (TVBC) and/or 7°C for 5 days (Psy.) Yousef and Carlstrom (2003).

### Statistical Analysis

The statistical analysis was carried out using ANOVA with one factor under significance level of 0.05 for the obtained results using SPSS and data were treated as a complete randomization design according to (Steel *et al.*, 1997). The experiments were performed in triplicate, using three samples per treatment (Siragusa *et al.*, 1999). Multiple comparisons were carried out applying the LSD test.

## Results and Discussion

### Physicochemical analysis and microbiological quality of beef and chicken fillets

Physicochemical properties and microbiological quality of beef and chicken fillet given in Table (1) show the moisture content was 73.48 and 74.37%, crude protein was 20.46 and 21.59%, ether extract was 4.76 and 2.83%, ash content was 1.1856 and

1.0483%, pH value was 5.77 and 5.8, TVN was (7.47 and 9.1 mg/100g), TBA was (0.263 and 0.169 mg MDA/Kg), total viable bacterial count was  $8.9 \times 10^3$  and  $7.2 \times 10^3$  CFU/g, and psychrophilic bacteria were  $8.8 \times 10^2$  and  $5.6 \times 10^2$  CFU/g in beef and chicken

fillets, respectively. These results confirm that beef and poultry rich in protein. These results are in agreement with those reported by Al-Najdawi and Abdullah (2002) and Salem *et al.* (2010).

**Table 1.** Physicochemical properties and microbiological quality of beef and chicken fillet.

Components*	Meat Types	
	Beef	Chicken Fillet
Moisture (%)	73.48±0.18	74.37±0.44
Crude protein (%)	20.46±0.8	21.59±0.08
Ether extract (%)	4.76±0.02	2.83±0.16
Ash (%)	1.1856±0.04	1.0483±0.02
pH value	5.77±0.03	5.8±0.03
TVN (mg/100g)	7.47±0.23	9.1±0.4
TBA (mg MDA/Kg)	0.263±0.014	0.169±0.011
Total viable bacterial count	$8.9 \times 10^3$	$7.2 \times 10^3$
Psychrophilic bacteria	$8.8 \times 10^2$	$5.6 \times 10^2$

\* Mean of triplicate determinations ±SE.

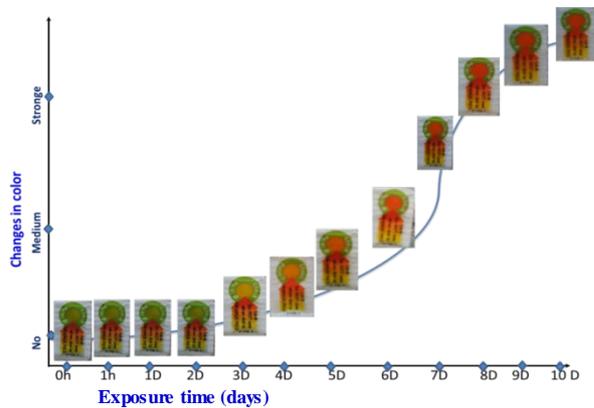
**Biosensor array for monitoring quality of beef and chicken fillets as smart packaging**

The design of the biosensor is based on the strong dye-analyte interactions, which is quite different from other electronic nose technologies that generally rely on weak, nonspecific intermolecular interactions, primarily van der Waals and physical adsorption interactions according to Fig. (1).



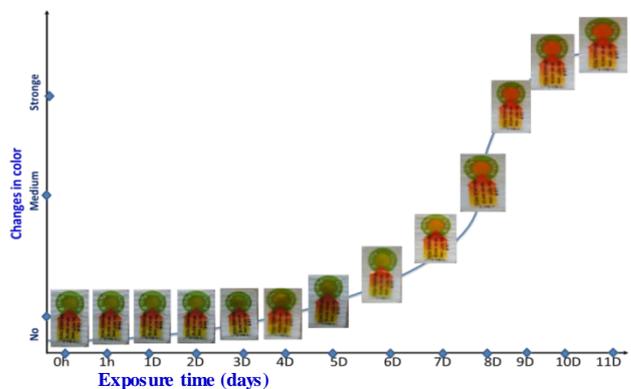
**Fig.1.** Biosensor array reactions.

As shown in Fig. (2) after 4 days of storage beef, no color change was observed in biosensor array its the same as of the original one (control), it still green color, this mean fresh beef, after that observed gradually color changes of biosensor array to orange color till 8 days of storage, this mean beef is still fresh and should use soon. After 9 days of storage there are strong color changes observed in biosensor array to red color, this mean beef not guaranteed.



**Fig.2.** Utilization of biosensor to monitor spoilage in package headspace of beef during storage at 4°C.

Regards of chicken fillets it noticed that after 6 days of storage, no color change in biosensor array, was nearly the same as that of the original one (control) it still green color this mean fresh chicken, after that observed gradually color changes of biosensor array to orange color till 9 days of storage, this mean chicken is still fresh and should use soon. After 10 days of storage there are strong color changes observed in biosensor array to red color, this mean chicken fillet is not guaranteed as illustrated in Fig. (3). The color changes of biosensor array due to volatile organic components (VOCs) emitted by the meat changed as a result of enzymatic activities taking place after the postmortem stage had started. At the early time of storage, the concentration of short-chain alcohols and hydroxyl group still low, then increased and microbes started to multiply. Metabolites such as amines were produced from the decomposition of protein due to microorganisms. Trimethylamine, TVBN and other amines gradually increased during the storage period. These obtained results generally are in agreement with those previously reported by Huang *et al.* (2011) and Pires *et al.* (2011).



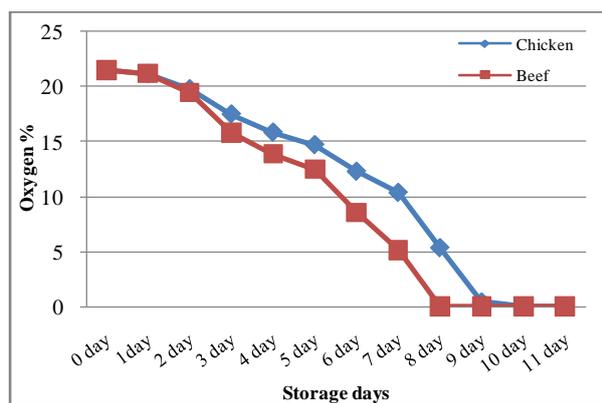
**Fig.3.** Utilization of biosensor to monitor spoilage in package headspace of chicken fillet during storage at 4°C.

### Application of oxygen sensor array to monitor quality of beef and chicken fillets

Oxygen is an essential element for all living organisms and also plays an important role in many chemical industrial processes, including those in which an absence of oxygen is required (Borchert *et al.*, 2012). It is also one of the most commonly analyzed chemical species, although many of the preferred detection methods, such as gas chromatography and electrochemical techniques, require rather expensive equipment and trained operators. Optical oxygen indicators are fast responding, inexpensive and easy to use (Kozak and Samotyja, 2013).

In recent years there has been dedicated non-invasive oxygen determination method developed based on fluorescence quenching of specific optical sensors sensitive to oxygen (Mills, 2005) and (Wolfbeis and Weidgans, 2006). The method was validated and implemented for common use in 2008 as ASTM F2714-08 standard.

Results illustrated in (Fig. 4) show the remaining oxygen percentage in the package headspace of beef and chicken fillet during storage at 4°C using oxygen sensor. It noticed that the remaining oxygen content in the package headspace of different samples such as beef and chicken fillet were 21.53 and 21.52%, respectively. Generally, remaining oxygen content in package headspace decreased continuously during storage at 4°C, it decreased from (21.53 to 0%) in beef sample after 9 days of storage, and (21.52 to 0%) in chicken fillet sample after 10 days of storage. The decrease in remaining oxygen content during storage might be due to activation of aerobic bacteria that consume the oxygen during decomposition of nitrogenous compounds. These obtained results generally are in agreement with those previously reported by (Papkovsky *et al.*, 2002).



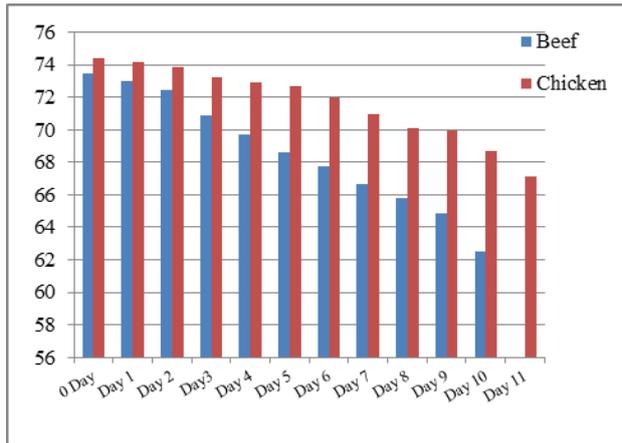
**Fig.4.** Oxygen sensor monitor O<sub>2</sub> percentage in package headspace of beef and chicken fillet during storage at 4°C.

### Physicochemical changes of beef and chicken fillets during storage

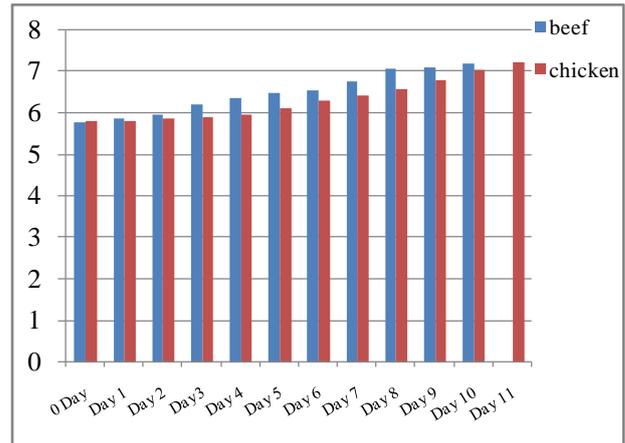
Data illustrated in (Fig. 5) show changes in the moisture content of beef and chicken fillets during storage at 4°C. Generally, moisture content of beef and chicken samples decreased continuously during storage period at 4°C. The initial moisture content in beef and chicken was 73.48 and 74.37%, respectively, while after ten days of storage, it was observed to be 62.52 and 68.72%, respectively. ANOVA indicated that there were significant differences ( $P \geq 0.05$ ) between samples in time zero and ten days of storage. The loss in moisture content of meat samples during storage may be due to the reduction of water holding capacity and increase the drip as a result of protein denaturation. In addition, evaporate of moisture through the polyethylene bags used to pack. These results are in agreement with those observed by (Rhee *et al.*, 2012).

The changes in pH value of beef and chicken fillets during storage at 4°C are illustrated (Fig. 6). The results indicate that the pH value of beef and chicken samples increased during the storage periods from (5.77 to 7.19) and (5.80 to 7.23), respectively. ANOVA indicated that there were significant differences ( $P \geq 0.05$ ) between samples in time zero and ten days of storage. The change of pH value was due to an increase in volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes. These results are in agreement with those observed by (Gheisari *et al.*, 2009).

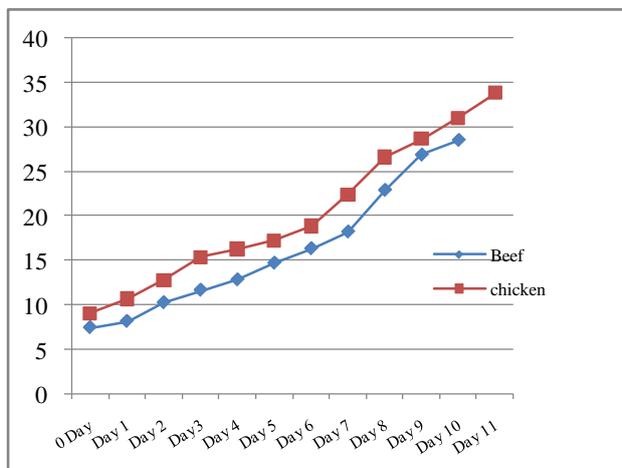
Total volatile basic nitrogen (TVB-N) and thiobarbituric acid (TBA) have been used as indices to assess the spoilage of refrigerated beef and chicken fillets. TVB-N was used for determination of the spoilage level of meat during the storage period. Total volatile nitrogen of beef and chicken fillets during storage at 4°C is shown in (Fig. 7). It noticed that TVN increased continuously during storage period (Sun and Holley, 2012). The initial TVN of beef was 7.47 mg/100g, while after ten days of storage, it was observed to be 28.47 mg/100g, whereas the initial TVN of chicken fillets were 9.10 mg/100g, while after ten days of storage, it was observed to be 33.83 mg/100g. ANOVA indicated that there were significant differences ( $P \geq 0.05$ ) between samples in time zero and ten days of storage. The level 25 mg/100g have been considered the upper limit, above which meats are considered spoiled (Gheisari *et al.*, 2009). Generally, the increase in TVN value during storage of meat samples might be due to rapid breakdown of protein through microbial decomposition. These results are in agreement with those reported by (Edris *et al.*, 2012).



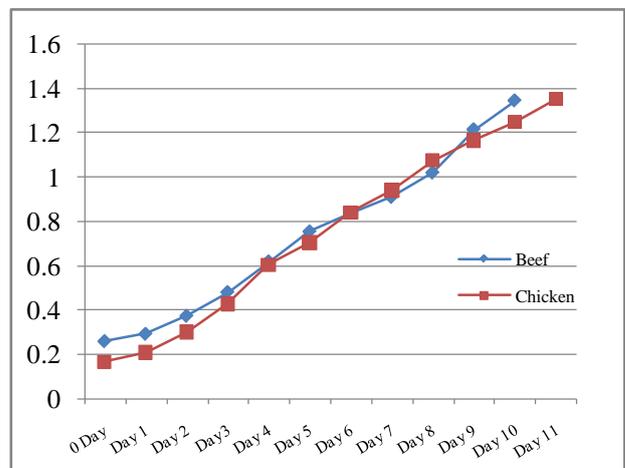
**Fig.5.**Changes in moisture content on beef and chicken fillets during storage at 4 °C.



**Fig.6.**Changes in pH value on beef and chicken fillets during storage at 4 °C.



**Fig.7.**Changes in the TVB-N on beef and chicken fillets during storage at 4 °C.



**Fig.8.**Changes in the TBA on beef and chicken fillets during storage at 4 °C.

Changes in thiobarbituric acid value during storage of beef and chicken fillets at 4°C were illustrated in (Fig. 8). The TBA values of beef and chicken fillets increased up to 10 and 11 days, respectively, depending upon storage time. The TBA results as the same trend in TVN. It increased from (0.263 to 1.347mg MDA/Kg) of beef, while on chicken fillets increased from (0.169 to 1.352 mg MDA/Kg). ANOVA indicated that there were significant differences ( $P \geq 0.05$ ) between samples in time zero and ten days of storage. These may be due to fat content which would speed up lipid oxidation. These results are in agreement with those observed by (Edris *et al.*, 2012).

**Microbial examination of beef and chicken fillets during storage**

The microbiological quality of beef and chicken fillets is dependent on a number of factors such as raw materials, sanitation during process and storage period. Data in Table (2) indicate that the total viable bacterial count (TBVC) of beef and chicken fillets was  $8.9 \times 10^3$  and  $7.2 \times 10^3$  CFU/g, while psychrophilic bacteria was  $8.8 \times 10^2$  and  $5.6 \times 10^2$  CFU/g,

respectively. The TBVC of beef and chicken fillets samples increased during the storage periods. It increased from  $8.9 \times 10^3$  to  $5.5 \times 10^7$  CFU/g of beef, while it increased from  $7.2 \times 10^3$  to  $7.1 \times 10^7$  CFU/g on chicken fillets. Also, psychrophilic bacteria increased from  $8.8 \times 10^2$  to  $9.1 \times 10^4$  CFU/g of beef, while it increased from  $5.6 \times 10^2$  to  $8.5 \times 10^4$  CFU/g on chicken fillets during storage periods at 4°C.

As recommended by the International Commission on Microbiological Specification for Food (ICMSF, 1986), an increase of total plate count (TPC) up to levels exceeding the value of 6 log CFU/g is regarded as microbial spoiled meat muscle not fit for human consumption. These results are in agreement with those observed by (Ohk and Bhunia, 2013).

**Table 2.** Microbiological quality of beef and chicken fillet during storage at 4 °C.

Storage period (Days)	Meat Types			
	Beef		Chicken fillet	
	TVBC	Psychrophilic	TVBC	Psychrophilic
0	8.9X10 <sup>3</sup>	8.8X10 <sup>2</sup>	7.2X10 <sup>3</sup>	5.6X10 <sup>2</sup>
1	1.14X10 <sup>4</sup>	9.4X10 <sup>2</sup>	7.9X10 <sup>3</sup>	6.5X10 <sup>2</sup>
2	5.6X10 <sup>4</sup>	1.12X10 <sup>3</sup>	1.04X10 <sup>4</sup>	7.9X10 <sup>2</sup>
3	6.3X10 <sup>4</sup>	1.24X10 <sup>3</sup>	1.27X10 <sup>4</sup>	9.2X10 <sup>2</sup>
4	7.8X10 <sup>4</sup>	5.1X10 <sup>3</sup>	4.4X10 <sup>4</sup>	1.03X10 <sup>3</sup>
5	9.2X10 <sup>4</sup>	7.1X10 <sup>3</sup>	6.9X10 <sup>4</sup>	5.3X10 <sup>3</sup>
6	1.13X10 <sup>5</sup>	8.7X10 <sup>3</sup>	9.2X10 <sup>4</sup>	7.0X10 <sup>3</sup>
7	4.1X10 <sup>5</sup>	1.03X10 <sup>4</sup>	1.05X10 <sup>5</sup>	8.9X10 <sup>3</sup>
8	5.7X10 <sup>5</sup>	1.15X10 <sup>4</sup>	1.19X10 <sup>5</sup>	1.02X10 <sup>4</sup>
9	5.7X10 <sup>6</sup>	6.8X10 <sup>4</sup>	7.6X10 <sup>5</sup>	5.4X10 <sup>4</sup>
10	5.5X10 <sup>7</sup>	9.1X10 <sup>4</sup>	9.6X10 <sup>6</sup>	6.7X10 <sup>4</sup>
11	ND	ND	7.1X10 <sup>7</sup>	8.5X10 <sup>4</sup>

Mean of duplicate determinations.

ND: Not detect

## Conclusion

In conclusion, new scientific disciplines and technologies have revolutionized the food sector. The results of this study have demonstrated that strong color changes were observed in biosensor array from a green color to red color that applied in beef and chicken fillet during storage, this mean these sample not guaranteed. Also, noticed that the remaining oxygen content in the package headspace of different samples decreased continuously during storage. Bio- and oxygen sensors are playing an important role in the formation of food processing technique and detection of spoilage and contaminants.

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## تطبيق المستشعر الحيوي و مستشعر الأوكسجين لكشف الفساد والتلوث البكتيري في اللحم البقري المفروم ولحم الدواجن المعبأة

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### الملخص العربى

تعتبر تكنولوجيا متناهية الصغر من التكنولوجيات المبتكرة في الآونة الأخيرة حيث تلعب دوراً جوهرياً في تحسين سلامة وجودة الأغذية، كما يعد إنتاج عبوات نانو ذكية تستطيع كشف فساد الأغذية هدفاً هاماً لأجهزة الرقابة على الغذاء وكذلك المستهلك. يعتبر المستشعر الحيوي و مستشعر الأوكسجين أحد تطبيقات النانو تكنولوجيا التي إكتسبت في الآونة الأخيرة الكثير من الإهتمام في مجال سلامة الأغذية. تضمن هذا البحث تطوير وتطبيق نموذجين من وحدات الإستشعار القادرة على التعامل مع عينات الأغذية المختلفة، حيث تم اختبارها على كل من لحوم البقر و فيليه الدجاج المخزنة على  $4 \pm 1^\circ\text{C}$  لمدة 11 يوماً. كما أجرى تقدير الخصائص الطبيعية، الكيماوية وكذلك أختبارات الطزاجة، والجودة الميكروبيولوجية على عينات اللحوم المخزنة على فترات منتظمة متزامن مع وحدات الإستشعار. أظهرت نتائج هذه الدراسة أن هناك تغير لوني قوي في المستشعر الحيوي حيث تحول من اللون الأخضر (طزاجة اللحم) إلى اللون الأحمر البرتقالي (فساد اللحم) بعد اليوم التاسع والعاشري كل من لحوم البقر و فيليه الدجاج، على التوالي. أيضاً أوضحت نتائج الاختبارات أن هناك زيادة في قيم كل من رقم الحموضة، النيتروجين الكلى المتطاير، حمض الثيوبارابوتريك خلال فترات التخزين. كما لوحظ أيضاً زيادة في محتوى العد الكلى للبكتيريا وكذلك البكتيريا المحبة للبرودة. أيضاً بينت الدراسة أن محتوى الاوكسجين المتبقي في عينات اللحم وفيليه الدجاج المخزنة إنخفض بشكل مستمر خلال فترات التخزين. هذه النتائج المتحصل عليها تقدم دليلاً واعداً لإمكانية تطبيق كل من المستشعر الحيوي و مستشعر الأوكسجين للكشف السريع عن فساد اللحم أثناء التداول والتخزين من أجل تأمين سلامة الأغذية.