Titre: Title:	Development of Films/Fibrous Nanostructures with Gas and Volatiles Detection Ability
Auteur: Author:	Dariush Karimi Alavijeh
Date:	2020
Type:	Mémoire ou thèse / Dissertation or Thesis
Référence: Citation:	Karimi Alavijeh, D. (2020). Development of Films/Fibrous Nanostructures with Gas and Volatiles Detection Ability [Mémoire de maîtrise, Polytechnique Montréal]. PolyPublie. https://publications.polymtl.ca/4221/

POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

Development of Films/Fibrous Nanostructures with Gas and Volatiles Detection Ability

DARIUSH KARIMI ALAVIJEH

Département de génie chimique

Mémoire présenté en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

Génie chimique

Février 2020

POLYTECHNIQUE MONTRÉAL

affiliée à l'Université de Montréal

Ce mémoire intitulé :

Development of Films/Fibrous Nanostructures with Gas and Volatiles Detection Ability

présenté par **Dariush KARIMI ALAVIJEH**

en vue de l'obtention du diplôme de *Maîtrise ès sciences appliquées*

a été dûment accepté par le jury d'examen constitué de :

Nick VIRGILIO, président
Abdellah AJJI, membre et directeur de recherche
Amir SAFFAR, membre

DEDICATION

To my family

ACKNOWLEDGEMENTS

I would link to extend my genuine thanks to those who helped me to accomplish this study.

Undoubtedly, my supervisor professor Abdellah Ajji deserves to be at the top of the list. I would like to express my appreciation to him for his patience and kindness. He always helped to put me in the right way, not only with his infinitive knowledge, but also with attitude for the quality of research.

I would like to extend my appreciation to Dr. Bentolhoda Heli for her support specially for her experience and knowledge transferred to me.

Special thanks to my friends and colleagues because of their support and friendship during my study.

Finally, special respect goes out to my family, for their support, encouragement and patience. More importantly, I would like to gratitude to my parents who have always been there for me. without their love, support and inspiration, this work could never have been done.

To my wonderful brother, Davoud for believing in me and encouraging me to reach the end of my ambition. Your hard work, your wisdom and your sense of discipline, make you a real source of inspiration. Thank you for being my brother

To my sister, for her support and love even though we were far apart.

Dariush Karimi Alavijeh

February 2020

Polytechnique Montréal

RÉSUMÉ

La détection colorimétrique de composés organiques volatils libérés lors de l'altération des aliments est l'une des méthodes les plus prometteuses pour indiquer le niveau de qualité d'un produit à base de viande lors de son conditionnement. Récemment, l'emballage intelligent a été introduit comme un outil fonctionnel prometteur dans l'emballage traditionnel et integrant des fonctions de communication. Il peut donc fournir des informations utiles sur la sécurité sanitaire et la qualité des aliments en surveillant l'évolution de l'environnement alimentaire. À cet égard, l'amélioration des techniques de détection a également fait progresser les emballages intelligents, ce qui permet aux clients de faire de meilleurs choix et d'éviter d'éventuels risques de maladie. Le capteur utilisé dans un emballage alimentaire doit être rapide, sensible, sélectif, peu coûteux et convivial.

Dans cette étude, nous proposons un capteur colorimétrique pour détecter la détérioration de la viande, fabriqué avec une plate-forme poreuse de nanofibres PLA enduites par immersion dans des anthocyanes comme colorant sensible au pH obtenu à partir d'une source naturelle (extrait de chou rouge). Lors de la détérioration de la viande, les composés azotés basiques volatils totaux (TVB-N) sont libérés à cause de la croissance des micro-organismes. Les quantités de ces composés, le triméthylamine (TMA), le diméthylamine (DMA) et l'ammoniac (NH3), sont des indicateurs importants pour évaluer la fraîcheur de la viande. En changeant le niveau de TVB-N dans l'espace présent dans l'emballage de la viande, la couleur du capteur passe du rose au bleu.

La plate-forme non poreuse a été préparée en utilisant la technique d'électrofilage dans différentes conditions pour obtenir le tapis nanofibreux le plus stable. Le mat résultant a été enduit par immersion dans diverses concentrations d'un colorant de pH extrait d'une source naturelle. Cela nous a permis de définir les conditions optimales du capteur proposé. Tout d'abord, la stabilité et la sensibilité du capteur développé ont été testées pour les composés TVB-N. Fait intéressant, il a montré une sensibilité élevée au TVB-N à diverses conditions de concentrations et de temps d'exposition. La couleur rose du capteur a été changée en bleu, vert et violet après exposition au NH3, au TMA et au DMA, respectivement, alors qu'elle n'a révélé aucune altération de couleur par exposition à d'autres composés organiques volatils tels que le formaldéhyde, le méthanol, le dimethylformamide, etc. Ensuite, la sensibilité du capteur a été examinée pour détecter la détérioration de la viande en contact direct avec les gaz produits pendant la détérioration. En

conséquence, il a montré un changement de couleur du rose au bleu et au violet avant le seuil de bactéries dans le bœuf (10^7 UFC / ml). Ce changement de couleur a été confirmé en comptant le total des micro-organismes viables. Par conséquent, il était parfaitement compatible avec le seuil de croissance des micro-organismes qui devait être considéré comme l'altération de la viande.

Le capteur proposé offre une sensibilité élevée grâce à sa structure unique. En outre, l'utilisation d'un colorant naturel peut être introduite comme sûre en raison de l'approbation de la FDA pour les capteurs applicables dans l'industrie de l'emballage alimentaire.

ABSTRACT

Colorimetric detection of volatile organic compounds in food spoilage is one of the most promising methods to indicate the quality level of a meat product during packaging. Recently, intelligent packaging has been introduced as a promising functional tool to the integration of traditional packaging and communication. Hence, it can provide useful information about food safety and quality by monitoring the changes in the food environment and its headspace. In this respect, the improvement in sensing techniques has also advanced intelligent packaging which leads to customers making better choices and avoiding possible risks of sickness. The applied sensor in a food packaging should be fast, sensitive, selective, low-priced and user-friendly.

In this study, we propose a colorimetric sensor to detect meat spoilage, fabricated with a porous platform of PLA nanofibers dip-coated into a anthocyanin as pH-sensitive dye obtained from a natural source (extracted form red cabbage). During the deterioration of meat, total volatile basic nitrogen compounds (TVB-N) are released by the spoilage due to the growth of microorganisms. These compounds, trimethylamine (TMA), dimethylamine (DMA) and ammonia (NH_3), are an important indicator in evaluating the freshness of meat. By changing the level of TVB-N in the head space of the meat packaging, the sensor color changes from pink to blue.

Accordingly, the porous platform was prepared using the electrospinning technique at different conditions to obtain the most stable nanofibrous mat. The resulting mat was dip-coated into various concentrations of a pH dye extracted from a natural source. This allowed us to define the optimum conditions of the proposed sensor. First, the stability and sensitivity of the developed sensor was tested for TVB-N compounds. Interestingly, it showed a high sensitivity toward TVB-N at various conditions of concentrations and exposure times. The pink color of the sensor was changed to blue, green and violet after exposure to NH_3, TMA and DMA, respectively, while it didn't reveal any color alteration by exposure to other volatile organic compounds such as formaldehyde, methanol, dimethyl formamide, *etc*. Then, the sensitivity of the senor was examined for the detection of meat spoilage being in direct contact with the produced gases during deterioration. As result, it displayed a color change from pink to blue and violet before the threshold of bacteria in beef (10^7 CFU/mL). This color change was confirmed by counting the total viable microorganisms. Therefore, it was

completely compatible with the threshold of the microorganism growth that should be considered as the spoilage in meat.

The proposed sensor offers a high sensitivity due to its unique structure. Besides, the use of a natural dye can be introduced as safe due to the FDA approval for sensors applicable in the food packaging industry.

TABLE OF CONTENTS

DEDICATION III

ACKNOWLEDGEMENTS IV

RÉSUMÉ V

ABSTRACT VII

TABLE OF CONTENTS IX

LIST OF TABLES XII

LIST OF FIGURES XIII

LIST OF SYMBOLS AND ABBREVIATIONS XV

CHAPTER 1 INTRODUCTION 1

1.1 Context 1

1.2 Objective 2

1.3 Plan of dissertation 3

CHAPTER 2 LITERATURE REVIEW 4

2.1 Introduction 4

2.2 Sensors 4

2.2.1 Chemical sensors 4

2.2.2 Fabrication of the colorimetric sensor 7

2.2.3 Nanofibrous colorimetric sensors 8

2.2.4 Applications of colorimetric sensors 11

2.3 pH sensitive dye 13

2.3.1 Anthocyanins 13

2.3.2 Chemical structure of anthocyanins 14

2.3.3 Extraction of Anthocyanin 15

2.4 Electrospinning....16
2.4.1 Poly lactic acid (PLA)....18
2.4.2 Application of electrospun fibrous mats....18
CHAPTER 3 EXPERIMENTAL METHODOLOGY....20
3.1 Introduction....20
3.2 Materials....20
3.3 Preparation of PLA nanofibrous mat through Electrospinning....20
3.4 Extraction of natural dye....21
3.5 Preparation of Colorimetric Sensor Platform....22
3.6 Volatile compound sensing and meat spoilage....22
3.7 Microbial test....23
CHAPTER 4 ARTICLE 1: DEVELOPMENT OF A SENSITIVE COLORIMETRIC SENSOR FOR DETECTING BEEF SPOILAGE IN SMART PACKAGING....25
4.1 Introduction:....26
4.2 Materials and method....29
4.2.1 Materials....29
4.2.2 Preparing the Electrospinning solution....30
4.2.3 Extraction of Anthocyanin....30
4.2.4 Fabrication of the Colorimetric Electrospun PLA Nanofiber Platform....30
4.2.5 Characterization of the Electrospun Nanofiber Mats....31
4.2.6 Evaluating the Performance of the Colorimetric Platform by exposing to ammonia 32
4.2.7 Sensitivity of the colorimetric sensor for detecting the Meat spoilage....33
4.3 Result and discussion....34
4.3.1 Characterization of nanofiber mats....34

4.3.2 Selectivity of the sensor through different VOCs 36
4.3.3 Examining the sensitivity of the colorimetric sensor toward ammonia 37
4.3.4 Colorimetric Sensing of Meat spoilage 42
4.3.5 Bacteria counting 43
4.3.6 Stability of sensor with humidity 44
4.4 Conclusion 45
4.5 Acknowledgments 45
CHAPTER 5 GENERAL DISCUSSION 51
CHAPTER 6 CONCLUSION AND RECOMMENDATIONS 53
6.1 Summary 53
6.2 Recommendations and Future Work 53
REFERENCES 55

LIST OF TABLES

Table 2.1 Different types of sensors.....6

Table 2.2 Colorimetric Sensors.....10

Table 2.3 Different Structure of anthocyanidins.....14

Table 2.4 Effect of electrospinning parameters on the morphology.....17

Table 3.1 Electrospinning specification.....21

Table 4.1 List of colorimetric sensors developed with natural dye.....28

Table 4.2 Comparison the properties of two different sensor platforms.....36

Table 4.3 Optical images of sensor before and after exposure to the moisture.....45

LIST OF FIGURES

Figure 2-1 Structure of anthocyanidin 14

Figure 2-2 Structure of anthocyanin in different pH.....15

Figure 2.3 Electrospinning setup.....16

Figure 3.2 Detection of Beef spoilage setup 23

Figure 4-1 Schematic of electrospinning 28

Figure 4-2 SEM images of a. Electrospun PLA nanofibers, b. the anthocyanin-impregnated PLA sample.....35

Figure 4-3 Selectivity of the sensor within the VOCs 36

Figure 4-4 (A) Optical image of sensor captured at different NH_3 concentration in the range of 1-100 ppm and (B) The corresponding calibration curve of the concentration and ΔRGB for dye concentration of (a). 0.57 (mg/mL), (b). 0.057 (mg/mL) and (c). 0.0057 (mg/mL) 38

Figure 4-5 (B),(D) and (F) show the variation of the RGB distance at the concentration of (a)100 ppm and (b) 1 ppm with respect to the dye concentration and (A), (C) and (E) shows the optical images of sensor as a function of detection time after the NH_3 exposures with dye concentration of 0.57 (mg/mL) , 0.057 (mg/mL) and 0.0057 (mg/mL), respectiv As reported earlier, to compare the sensitivity of the PLA electrospun nanofiber mats with other substrates, the bacterial cellulose also was applied as a solid matrix support in the sensor. Figure 4-6 A. and B. reveals the results of NH3 detection and comparison of different substrates. As it is clear, PLA porous media represent better results in terms of sensitivity at a higher NH3 concentration (100 ppm), which is probably due to its high porosity and surface area. However, in a lower concentration of NH3 (1 ppm), the colorimetric results are almost at the same range and this is probably because at lower concentration of NH3, the kinetic reaction is independent from porosity. Another limitation of the bacterial cellulose for a colorimetric sensor application is its transparency and visual properties when compared with polymeric electrospun medias. 40

Figure 4-6 RGB distance as a function of NH_3 exposure time (A) 100 ppm (B) 1 ppm for (a) PLA electrospun mats and (b) bacterial cellulose as a substrate 41

Figure 4-7 Colorimetric analysis and optical image of sensor during the beef spoilage for (A) room temperature (23 ºC) in 24h and (B) for 4 ºC in nine days 43

Figure 4-8 TVC count during storage (A) 23 ºC followed up to 24 h and (B) at 4 ºC followed up to nine days.....44

LIST OF SYMBOLS AND ABBREVIATIONS

VOCs Volatile organic compounds

TMA Trimethylamine

DMA Dimethylamine

TVB-N total volatile base nitrogen

NH_3 ammonia

µPADs microfluidic paper-based analytical devices

PMMA Polymethyl methacrylate

PDPA Poly diphenylamine

PANI Poly aniline

HCSA hydroxylated cyclosporine

PAA Poly Acrylic acid

TCFH Tetramethyl-chloroformamidinium hexafluorophosphate

BCG bromocresol green

PS Polystyrene

PVA Poly vinyl acetate

PCL Poly caprolactone

PEO Poly ethylene oxide

MR Methyl Red

PDA Polydiacetylenes

Au Gold

Cu Copper

Br-PADAP 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol

TVC total viable bacterial counts

Pg	Pelargonidin
Cy	Cyanidin
Pn	Peonidin
Dp	Delphinidin
Pt	Petunidin
Mv	Malvidin
HCl	hydrochloric acid
CO_2	carbon dioxide
Fe	Iron
PLA	Poly lactic acid
PM	pyrene methanol
TFE	Trifluoroethanol
H_2O	Water
EtOH	ethanol
PBS	phosphate buffered saline

CHAPTER 1 INTRODUCTION

1.1 Context

Protein-based food, especially meats, have always been the main source of energy for mankind that may be consumed either raw or cooked. Meat spoilage is a metabolic process that may or may not cause illness because there might or might not be pathogens or contamination present. But, changes in texture, taste and appearance cause them to become unacceptable for human consumptionnon [1].

Recently, strategies for real time quality monitoring and for the increase of the shelf life of packaged food is in demand by manufacturers and consumers. Active packaging technology has been used to change the condition of packed food in order to improve safety and extend its shelf life. Active materials such as oxygen scavengers, antimicrobials and antioxidants are used in the surrounding environment of food products to improve its shelf life when packaged. However, active packages do not provide any information about packaged food quality or at the point of consumption. Monitoring the freshness and appropriateness of food for comsumption is important in order to decrease food waste and food poisoning. Accordingly, Smart packaging is employed to carry out such tasks. This packaging is proposed to detect, sense and record any deterioration in food packaging in order to improve the safety and quality of food during its storage [2]. Smart packaging employs chemical sensors or biosensors to monitor the quality and safety of food from production to consumption. This technology leads to a variety of sensor designs incorporating aspects such as freshness indicators as well as detection of pathogens, carbon dioxide, oxygen, pH and temperature fluctuations [3].

In recent years, numerous efforts have been directed toward the manufacture of freshness indicators, including colorimetric sensors in food packaging applications [4-6]. organic compounds are produced during the storage of protein-based products such as meat and poultry due to the deterioration of its protein chains by microorganisms. In such cases, the spoilage microorganism breaks down the protein chains into amino acids and this phenomenon, produce amine-based compounds including Trimethylamine (TMA), Dimethylamine (DMA) and ammonia (NH_3). These compounds are also known as total volatile base nitrogen (TVB-N) and their concentration increase gradually in the head space of the food package over time [7].

Various kinds of detectors are being currently investigated to determine the quality of meat products. Among them, colorimetric sensors are promising because of their advantages such as the ease to read, low cost, ease of manufacturing, *etc*.[8]. PH sensitive dyes are mostly used as a sensing element in colorimetric sensors due to color change upon exposure of the detectable analyte such as NH_3 due to the acid based reaction occurring between sensing element and the analyte [9, 10]. Additionally, employing pigments extracted from natural sources have advantages such as low toxicity over other pigments acquired from other sources [11]. Similarly, anthocyanins is a group of natural pigments that are widely distributed in plants, mostly in red colored vegetables and flowers [12]. The halochromic property of these pigments make them a proper alternative for conventional dying usually used in the fabrication of colorimetric sensors.

Many studies have been reported on the development of porous nanofiber structures from different polymers by various methods including electrospinning [4, 5, 13]. Electrospinning is one of the conventional methods to fabricate a non-woven nano porous fiber mats with a large surface area to volume ratio by applying an electric field between a polymer solution and a collector plate [6]. This method allows colorimetric sensors to provide higher sensitivity due to the mats high surface area [14]. The higher surface-to-volume ratio of platform leads to a higher sensor sensitivity. Polylactic acid (PLA) nanofiber mats fabricated with the electrospinning process possesses [15] properties of interest as a solid support matrix for the immobilization of the dye.

As a problem identification, there is a need for developing the Fast, sensitive, selective, safe user-friendly sensor. Also, developed sensor should be applicable for food packaging in order to detect and monitor the freshness of food specially meat through changes in its headspace environment

1.2 Objective

The objective of this research is to develop a highly sensitive platform functionalized with a natural dye-based pH-indicator for the detection of NH_3.

to explore the performance of the developed platform for the detection of TVB-N in meat spoilage and its applicability in the food packaging industry.

1.3 Plan of dissertation

This dissertation comprises of six chapters. Following this chapter, Chapter 2 will present a brief literature review of sensors including their definition, fabrication techniques and applications. Chapter 3 will describe the applied methodology: experimental plan, equipment and facilities that are implemented in this work. Chapter 4 will describe obtained results of this study in the form of a scientific article. General discussion and conclusions/recommendations will be presented in chapters 5 and 6, respectively.

:

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This chapter discusses operating principles and design systems of a variety of chemical sensors with specific focus on colorimetric sensors. The fabrication and application of electrospun nanofibers as a colorimetric platform are discussed in detail. Additionally, the chemical structure and the mechanisms of halochromic dyes particularly anthocyanins as a pH sensitive dye are discussed further. Parameters that were varied to investigate changes in sensitivity of the colorimetric sensor are also presented.

2.2 Sensors

Sensors are devices that can detect and identify the presence of various substances or chemical constituents such as toxic analytes. Many researchers have focused on developing sensors with high sensitivity, reversibility, stability, reproducibility and selectivity for identification and detection of a various range of materials such as toxic gases, vapors and aqueous solutions [16, 17].

Persaud *et al.* have presented one of the first sensors using a semiconductor transducer to detect a wide variety of odors [18]. Different kinds of materials such as polymers, semiconductors, carbon graphite, halochromic dyes and nanoparticles have been used in order to develop a sensitive sensor that can detect various types of hazardous analytes [19-23]. So far, different types of sensors have been successfully investigated for distinguishing different kinds of toxic and hazardous compounds. Table 2.1 provides the different types sensors, with a focus on optical sensors.

In the last decade, there has been a tremendous increase in demand for sensors. This has resulted in extensive research on their development especially chemical sensors.

2.2.1 Chemical sensors

There is a critical demand on measuring and monitoring the chemical environment. The impact of the environment on human health is a major concern where the analysis and amounts of a range of toxins and pollutants becomes of increasing significance. Such analyses need small and inexpensive sensors to convert an input signal into a readable output signal. The input signal can

be any measurable characteristic (quantity or physical variation) while the output should be any readable signal [24]. Chemical sensors respond to the chemical environment and can be categorized into the thee major groups: electrochemical, thermometric and optical sensors. In this dissertation, our focus is on the last category, but we briefly reviewed all the sensor categories.

2.2.1.1 Electrochemical sensors

An electrochemical sensor is a resistive device that transforms electrochemical information into a readable signal. Electrochemical sensors usually comprise of two components, a recognition system, that chemically interacts with the chemical analytes and physicochemical transducer which is a device that converts the chemical response into a signal that can be monitored by special instruments. These kinds of sensors have various applications in food, pharmaceutical, bio sensing and environmental industries [25].

2.2.1.2 Thermometric sensors

The mechanism of this type of sensor is based on differences in temperature resulting from chemical reactions or adsorption phenomena. The thermometric sensor is made by a catalytic sensor layer which is coated on the surface of a thermometer. In this case, the interaction between target analyte and sensor layer can consume or generate heat which in turn can be detected and/or measured by a sensitive thermistor. The thermometric sensor has different applications such as the detection of sucrose, glucose, uric acid *etc*.[4, 26]

2.2.1.3 Optical sensors

Optical sensors are composed of analytic tools which provide the information about target analytes via visible or ultraviolet light. Different techniques have been used to study the presence of target analytes for example; refractive index, scattering, diffraction, absorbance, reflectance, photoluminescence, chemiluminescence, *etc.* In this review, our focus is on an optical sensor that uses absorbance and the reflectance technique.

The advantages of optical indicators have led to their development as a suitable alternative to other types of sensors as they provide a facile and efficient approach for rapid detection of target substances. An optical sensor contains a detector agent that interacts, either with chemical reaction

or physical adsorption, with the desired analyte, resulting in colorimetric or fluorescent changes on a solid substrate support.

2.2.1.3.1 Colorimetric sensors

Colours have been used from many years as universal indicators. For example, red and green are the signs for stop/danger and go/safe respectively. Since colors are so easy to detect visually, they represent the great signal for monitoring the circumstances and systems such as sensors. Quantitative measurement of absorbance-reflectance spectra (colorimetric analysis) is one of the oldest techniques for detecting chemical analytes. Many types of colorimetric sensors have been developed and it is still being researched. For instance, the first colorimetric sensor array was presented by Suslick *et al.* which, was not only capable of detecting the analyte, but also provided information about different compounds [27].

The color changing agents have been immobilized on various types of substrates. Martinez *et al.* demonstrated microfluidic paper-based analytical devices (μPADs) for the colorimetric detection of glucose and protein [28]. Ajay *et al.* described the different polymeric thin films incorporated in colorimetric sensors for various types of analytes [29]. Bacterial cellulose also has been used to develop the colorimetric platform due to its porous structure. There are many reports on the using of bacterial cellulose as solid substrate incorporated in the sensor[30-32]. Furthermore, other types of substrates are applied to develop colorimetric platforms [33-38]. Among all these colorimetric platforms, nanofibers show a great potential as a colorimetric platform due to its specific advantages such as high surface-to-volume ratio.

Table 2.1 Different types of sensors

Type of sensor	Materials	Target analytes	Limit of detection	Ref:
thermometric	porous glass bead-enzymes Hexokinase Uricase	Sucrose glucose uric acid	0.1-50 mM 0.5–25 mM 0.05–4	[4] [26]

	β-Lactamase	Penicillin G	0.001–200	
electrochemical	PDPA-Polymethyl methacrylate (PMMA) Poly aniline (PANI) PMMA-PANI HCSA-PANI Co-ZnO	NH_3 Amines $(C_2H_5)3N$ Alcohols O_2	1 ppm 100 ppm 20 ppm - 0.32 Torr	[39] [13] [40] [41] [42]
optical	Poly Acrylic acid (PAA)-TCFH PAN-BCG Polystyrene (PS)-Tetraphenylporphyrin Cellulose acetate- Py 1 PCL- Methyl Red &rose Bengal PVA-Ag NP PLA-Tetraphenylporphyrin PEO-PDA	NH_3 NH_3 HCL Biogenic amines VOCs Biogenic amines HCL CO_2	- 10 ppm 1ppm 1ppm 10ppm 100ppm	[6] [43] [44] [45] [46] [47] [48] [15]

2.2.2 Fabrication of the colorimetric sensor

During past decades, various techniques have been developed to fabricate a sensitive and selective platform. Usually, colorimetric sensors comprise of two main parts: substrate and recognition agent. Different types of solid substrates are used to carry the detector agents. For example, paper

is one of the ancient substrates in designing a colorimetric sensor. Gay-Lussac described the litmus paper test for acids early in 1800s [49]. Due to the surface area and capillary action paper is one of the most conventional substrates in colorimetric sensors [34]. However, paper is insufficient in some application because of its mechanical properties and wettability. In other research, sol-gel thin films are also recognised as a promising strategy to produce both colorimetric sensors and bio sensors. Using the sol-gel technology as an alternative to conventional glass due to its advantages as its enzyme immobilization matrices are superior. These advantages include high thermal and chemical stability, simplicity of preparation and ability to control the pore size [36].

Moreover, some polymeric material also incorporates electrospun nanofibers into colorimetric sensor application as a solid matrix. Electrospun nanofibers have been in great demand in sensor applications owing to its great sensitivity. Wang *et al*. have shown that the sensitivity of electrospun nanofibers is greatly improved due to the higher surface area-to-volume ratio than thin film sensors [50]. In this review, the fabrication of electrospun nanofibers and their application are discussed further below.

2.2.3 Nanofibrous colorimetric sensors

As mentioned before, electrospun nanofibers are employed as a solid media to carry the colorimetric agents (Table 2.2). Halochromic dyes and plasmonic nanoparticles (Ag and Au) are mostly used as a colorimetric agent to detect and show the presence of hazardous analytes by changing its color. To introduce these agents to nanofibrous material thee different techniques are typically used; dye-doping, dip coating and drop casting [35].

2.2.3.1 Dye doping

Currently, dye-doping is one of the most common techniques for making colored nanofibers during electrospinning, which supply a simple way to produce halochromic nanofiber by adding the colorimetric agent to the electrospinning solution prior to electrospinning. This method could prevent dye leaching by using a covalent linkage between the polymer matrix and colorimetric agent. However, this method can affect the sensitivity of the sensor [46, 51, 52]. Liang *et al.* have researched on the optimization of the dye concentration during the production of the nanofibrous membrane using a dye-doping technique for the detection of biomolecules. They found that the

sensor performance is not only affected by concentration of the dye but, also the fiber diameter and pore structure which are also important parameters in the sensor performance [53]. Although, dye-doping is a conventional method to immobilize colorimetric agents into the nanofibrous matrix, this method has some challenges reviewed by Frenot *et al.* [54].

2.2.3.2 Dip-coating

Dip-coating is another type of fabrication technique for making a colorimetric platform. In such a method, a nanofibrous mats are completely immersed into the dye solution to absorb a halochromic agent onto its surface [55]. In this type of immobilization, a halochromic agent (which are pH sensitive dyes or plasmonic nanoparticles) is attached physically to the substrate and this can lead to the dye leaching. Some research has been done regarding the application of the dip coating technique in order to develop the colorimetric sensors. For instance, Wang *et al.* investigated the sensitivity of the PAN/fluoral-p colorimetric sensor strips using the dip-coating method [55]. Schmitt and his team also prepared a colorimetric gas sensor to detect various toxic gases by dip-coating [56].Gong *et al.* used the dip coating technique to fabricate graphene oxide thin film for colorimetric sensing [37].

2.2.3.3 Drop casting

For small substrates ($\leq 1 cm^2$), drop casting is the perfect technique. In such method, a droplet of solution spread over the substrate surface and is allowed to dry under controlled conditions (temperature and pressure). Drop casting does serve a rapid and accessible method to generate the colorimetric platforms applicable for various type of detection [57].

Generally, in this method, it is desirable to use highly volatile solvents because it could damage the morphology of the substrate. For example, water (H_2O) is not a suitable solvent in drop-casting due to the low vapor pressure and large surface tension. For this method organic solvents such as hexane, toluene and alcohol are preferred choices [58].

Table 2.2 Colorimetric Sensors

Nanofiber matrix	Recognition agent	Analyte	Limit of detection	Ref:
Poly amide	Cresol Red p-Rosolic Acid Bromothymol Blue Bromocresol Purple Neutral Red Chlorophenol Red	pH	-	[59]
	Au/Cu	Ascorbic acid	$18\times10^{-3}g.L^{-1}$	[60]
PCL	Nitrazine Yellow	pH	-	[61]
	Dimethylglyoxime	Ni^{2+}	1ppm	[62]
PAN	Pyran-derivative	pH	-	[63]
Cellulose acetate	Br-PADAP	Uranyl	50 ppb	[64]
Zain	Curcumin	Fe^{3+}	0.4 mg L^{-1}	[65]
		pH	-	[66]
PEO	o-phenylenediamine derivatives containing rhodamine, 1,8-naphtalimide	Phosgene	0.7 ppb	[67]

	and 4-chloro-7-nitrobenzo			
PAA	Hydrazone-tricyanofuran	NH_3	1.1×10^{-6} $mol.L^{-1}$	[6]
PLA	Tetraphenylporphyrin	HCL	34 ppb	[48]
PU	ABTS	Glucose	5×10^{-8} mol	[68]
PVA	Cu	Glyphosate	$1\times10^{-7}g.L^{-1}$	[69]
Silicon oxide	MR Methyl Yellow	HCL, NH_3, biogenic amines	20 ppm	[70]

2.2.4 Applications of colorimetric sensors

Colorimetric sensors have been accepted as a highly sensitive and selective platform for the detection of various analytes. Hence, it has a lot of applications in different fields such as waste water industries, petroleum industries and food industries. In this review, colorimetric sensors for the detection of organic compounds and specifically its application in food packaging have been elaborately discussed.

2.2.4.1 Detection of total volatile compounds

Many industrial activities such as in the petroleum industry, have emitted the toxic pollutants to the atmosphere including VOCs. Moreover, almost every human activity such as cooking, driving, manufacturing results in the emission of VOCs such as alcohols, alkanes, alkenes, esters, aromatics, ethers, and amides [71]. Some of these compounds have been identified as highly toxic and may have both short-term and/or long-term effects on human health. NH_3 gas is a well-known example. NH_3 in form of gas or liquid can cause numerous diseases such as asthma and lung cancer even in low concentrations.

Colorimetric sensor has the potential to be powerful detectors of chemically diverse analytes such as volatile organic compounds. Earlier, the electric nose technique was mostly used for the detection of VOCs. However, as electric nose is less sensitive for detection of VOCs in low concentration and it needs additional devices such as transducers [72].Therefore, a colorimetric sensing platform should be considered a proper alternative which overcomes the above drawbacks.

2.2.4.2 Food spoilage

In recent years the consumption of protein-based products particularly beef has increased in many countries around the world, which brings the concern of freshness to the focus among its costumers. Hence, recent incidents of food borne diseases have become a serious problem. Since there have not been suitable devices for monitoring the level of quality, it's apparent that food borne diseases might be the reason for poor quality control. Until now, total viable bacterial counts (TVC) and total volatile basic nitrogen (TVB-N) are the major methods in order to evaluate meat quality in the meat production industries [73]. Moreover, other methods take several days to determine the level of the quality of meat which means that they are not helpful in evaluating the freshness of the meat products that are currently being sold [74]. The TVB-N content in beef, as an important reference element, has been used to evaluate freshness of meat freshness [75]. TVB-N consists of thee diverse compounds TMA, DMA, NH_3 in which their levels are dependent on the activity of the living microorganisms during the deterioration of meat [7]. However, this method is extremely time consuming, expensive and also not practical to apply in food packaging [76]. TVC is one of the other methods of detection of spoilage in protein-based foods. In this method, the bacterial colonies growth is counted, and the results determine the spoilage of meat. Based on the literature, the threshold of bacteria to be considered as spoiled is 10^7 CFU/mL [77]. But this method is time consuming and needs special equipment.

Therefore, the quality control and the freshness indicator in meat packaging demands a development of an inexpensive, simple, rapid, handy and accurate sensor.

2.3 pH sensitive dye

Since pH changes are an substantial factor in determining the spoilage of meat in food products, many efforts have been made though the development of visual colorimetric indicators using pH sensitive dyes due to their advantages including low cost and good sensitivity [78].

Brønsted acidic or basic dyes (pH indicator dye) are halochromic chemical compound, which is sensitive to the concentration of hydrogen ions. Therefore, they can be used as an indicator in different pH values. There are a huge number of pH indicators for example; cresol red, methyl red, bromocresol purple and phenol red [79], although for food packaging applications, the use of these materials is avoided due to their toxicity and potential harmful effects to human beings [80]. Recently, several studies have focused on potential of pH sensitive dyes from natural sources such as the colorimetric quality in sensors for intelligent food packages [81], Subsequently, pH sensitive dyes obtained from natural sources are pollution free, easy to extract and non toxic. Among the different types of plant-based natural dyes, anthocyanin has gained a lot of attention because of sensitive color reaction to a broad range of pH (2 - 9) [38]

2.3.1 Anthocyanins

Anthocyanins are one of the largest categories in phenol compounds. The basic structure of anthocyanins comprises of the flavylium cation which could be attached to different groups of chemical structures such as sugars, hydroxyl, metaxyl, *etc.* Furthermore, this combination could lead to a variety of anthocyanins which has been known so far [82].

The color and structure stability of anthocyanins are dependent on temperature, light, enzymes, and inter/intra molecule corporation with different compounds such as metal ions and proteins [83]. The appearance of anthocyanins describe a reversible change in color in different pH values from red (pH =2) to blueish green(pH=9) in acid and base environments respectively[84].

2.3.2 Chemical structure of anthocyanins

Anthocyanidin is the basic structure of anthocyanins which comprise of an aromatic ring attached to a heterocyclic ring which is bonded to another aromatic ring (Figure 2.1). The different structure of anthocyanidin is shown in Table 2.3.

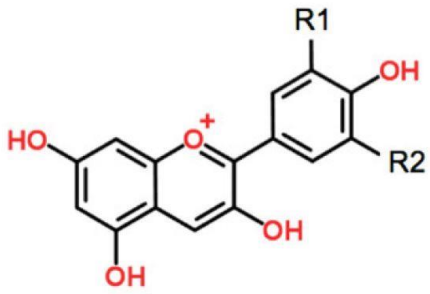


Figure 2-1 Structure of anthocyanidin

Table 2.3 Different Structure of anthocyanidins

Name	R1	R2	Abbreviations
Pelargonidin	−H	−H	Pg
Cyanidin	−OH	−H	Cy
Peonidin	$-OCH_3$	−H	Pn
Delphinidin	−OH	−OH	Dp
Petunidin	$-OCH_3$	−OH	Pt
Malvidin	$-OCH_3$	$-OCH_3$	Mv

As previously reported, anthocyanins have shown a sensitive color reaction for a wide range of pH (red color in acid surround and bluish green in base surround). Anthocyanins can be found in different structural chemical forms (Figure 2.2) which depends on the pH of the solution. This feature of anthocyanin has gained a lot of attention in the past few years for developing a novel

colorimetric sensor that is safe and non toxic to indicate the quality of the freshness in food application.

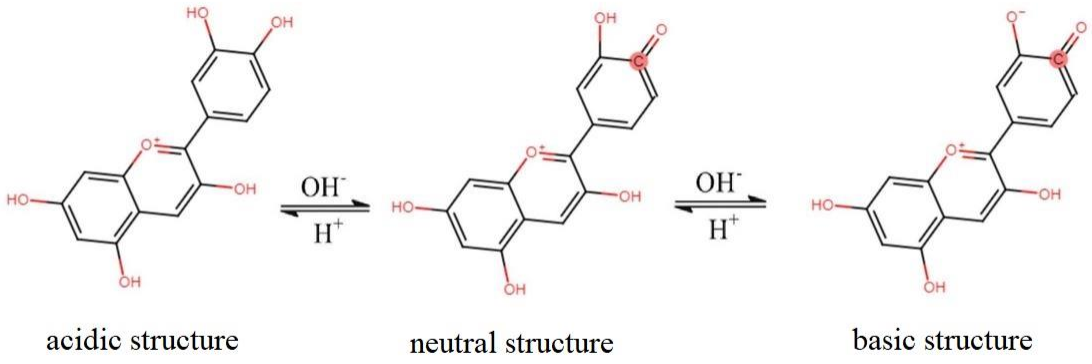


Figure 2-2 Structure of anthocyanin in different pH

2.3.3 Extraction of Anthocyanin

Anthocyanins as natural pigments are widely distributed in plants such as red cabbage, sweet potato, grapes, berries, flowers, *etc.* [85]. Anthocyanins extracted from various natural sources may have different concentrations which is leads to a difference in intensity and colour stability [86]. For instance, solid-liquid extraction is a promising method to extract compounds by solvent [87]. Polar solvents have shown the better results during the extraction of anthocyanins due to the polarity of the anthocyanin structure. On the other hand, some of these solvents such as acetone and sulfur are toxic and harmful for human health [88].

Among the different polar solvents, methanol and ethanol are the most efficient extractants [89]. Metivier *et al.* have demonstrated that the extraction of anthocyanin from grape pulp with methanol is 20% more effective than ethanol and 70% more effective than H_2O alone [90]. Nevertheless, ethanol is preferable the methanol due to the lower toxicity. Some studies have found that acidified solvent could improve the efficiency of extraction and stability of the extracted pigments[91, 92]. However, extraction with an acidified solvent could degrade the hydrolysis reaction in the anthocyanins[89].

Anthocyanin extracted from red cabbage, (*brassica oleraceae)*, exhibit color over a very broad range of pH from red at low pH to blue and green at high pH. Unlike, other sources of anthocyanin such as grape skin and sweet potato which provide a reasonable range of color at only pH<4. Including low cost of red cabbage compared with other sources, anthocyanin extracted from red cabbage has become attractive in the application of visual and colorimetric indicators.

2.4 Electrospinning

It is worth mentioning that the sensitivity of sensors is truly affected by the surface-to-volume ratio of sensing platform. The higher surface-to-volume ratio of platform leads to a higher sensor sensitivity. Hence, many techniques have been used to increase the surface-to-volume ratio [93-96]. The electrospinning technique is known as a conventional method to fabricate porous media with a high surface-to-volume ratio.

This method is used to fabricate nano porous fiber media with an applied electric field which causes the random formation of micro-to-nano scale fibers. In a common electrospinning setup (Figure 2.3), the polymer solution in plastic or glass syringe is electrospun to the grounded conductive collector plate by using a stainless-steel spinneret that is attached to the positive side of high voltage power supply. When the voltage reaches the critical value, the polymer solution is hurled as a stream of fibers beaded into the Tylor cone by means of a force applied by the infusion pump. Then, the rapid evaporation of the solvent helps the solidified fibers to be formed randomly onto the collector plate and forms the nonwoven fiber mat.[97-99]

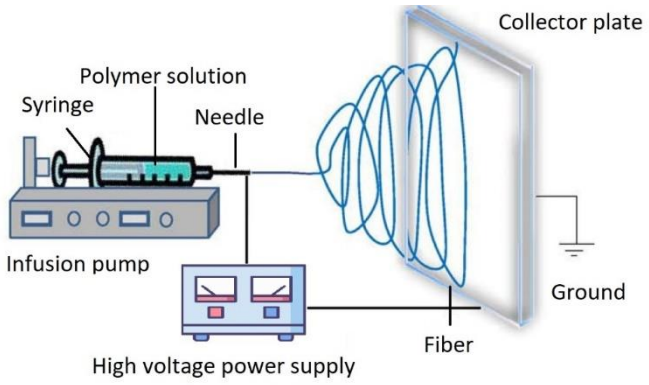


Figure 2.3 Electrospinning setup

There are several parameters that can affect the morphology of the fabricated nano fibers including the applied electric field, tip-to-collector distance, needle diameter size, polymer concentration in the electrospinning solution, solution conductivity, viscosity and even environmental parameters such as, temperature and relative humidity [100]. Table 2.4 describes the effect of each parameter on the fibers.

Table 2.4 Effect of electrospinning parameters on the morphology

Parameter	Effect of fibers	Ref:
Applied voltage	Increasing the applied voltage results in decreased stability of solution jet, increases the number of beads and decreases the fiber diameter	[101]
Concentration of polymer	Decreasing the solvent, increases the number of beads	[102]
Tip-to-collector distance	Larger the distance, increases the number of beads and decreases the fiber diameter	[103, 104]
Needle diameter	Increasing the needle diameter, increases fiber diameter	[105]
Solution conductivity	Increasing the solution conductivity, decreases the fiber diameter	[106]
Viscosity	Increasing the viscosity, results in larger fiber diameter and larger beads	[107]
Temperature	Increasing the temperature, decreases the viscosity and fiber diameter	[108]

Relative humidity	Increasing the humidity, results in thinner fibers and increases the number of beads	[109, 110]

Some of these parameters have more influence on the fiber morphology and their characteristics. One of the most important factors which has a significant effect on the diameter of electrospun fiber is viscosity, which depends on the polymer molecular weight and its concentration in the electrospinning solution[107]. For instance, by increasing the viscosity of solution, the obtained fiber diameter becomes larger and their morphology changes from rotund to spindle-like.

The applied voltage also plays an essential role on the morphology of fibers. Many studies have reported that a smaller size of fiber diameter is achieved with increasing the applied voltage [101].

The distance between the tip and collector plate has an important effect in determining the final morphology of the nanofibers and it can be varied based on its deposition time, evaporation rate of solvent and wiping time [103]. However, in most of cases, increasing the distance between needle tip and collector leads to smaller diameter fibers.

2.4.1 Poly lactic acid (PLA)

PLA has been widely employed in various applications specifically in for bio medical and tissue engineering due to it bioresorbable and biodegradable properties. Furthermore, PLA can be easily fabricated via the electrospinning technique. Regarding the high porosity as well as large specific surface area, PLA electrospun nanofibers were chosen in this research as the best candidate for a gas sensor carrier matrix.

2.4.2 Application of electrospun fibrous mats

Nano materials in the form of fibers obtained from the electrospinning process has gained a lot of attention in this century although Formhals presented the first method of electrospinning in 1934. Due to the large surface area and ease of process, electrospun fibers tend to become one of the most promising materials in various fields for applications such as biosensors, enzyme immobilization, tissue engineering, wound dressing, drug delivery, *etc*.[111-114]. Stizal *et al*. have reported the use

of electrospinning for the fabrication of scaffolds in tissue engineering[115]. Furthermore, Woo *et al.* have successfully investigated the usage of electrospun nanofibrous scaffolds to promote cell growth and protein adsorption [116]. Besides the numerous applications of electrospun nanofiber mats, they have also gained lots of attention in sensor applications. Wang *et al.* first have worked on electrospun PAA grafted with pyrene methanol (PM) nano fibers as a sensor for detection of metal ions Fe^{3+} and Hg^{2+} in H_2O [50]. In addition, in other work, PM-grafted PMMA nanofibers were used to detect 2,4-Dinitrotoluene (DNT) [117]. Electrospun PAA nanofibers were used to detect and determine the level of NH_3 gas in ppb level. This platform was used due to the high surface area of the electrospun mats which had a sensitivity of four times higher than that of PAA cast film [118]. Electrospun polymeric nanofibers consisting of pH sensitive dyes have shown sensitivity and selectivity towards VOCs. When the concentration of organic compounds reaches its threshold, the sensitive dye changes their initial color.

In this study, a similar electrospinning method has been adapted to produce nanofibrous mats as a carrier for natural-based, pH sensitive dye as an optical sensor for detection of TVB-N in protein-based food applications.

CHAPTER 3 EXPERIMENTAL METHODOLOGY

3.1 Introduction

This chapter describes the experimental methodology used to fabricate the colorimetric sensor And, then, the analysis technique of the sensor is discussed . The list of materials along with their suppliers used to proceed this project are listed here, as well.

3.2 Materials

The PLA (grade 4032D with molecular weight M_w=133,000) was obtained from Nature Work LLC. Bacterial cellulose was obtained from Nanonovin Polymer Co. (Mazandaran, Iran). Ammonium hydroxide (30-33% NH_3 in H_2O), 2,2,2-Trifluoroethanol (TFE, ACS reagent, ≥99%), TMA (anhydrous, ≥99%), DMA (anhydrous, ≥99%), ethanol (EtOH, ≥99.9%).), hydrochloric acid (HCL) and filter paper (Whatman NO.1) were supplied from Sigma Aldrich (ON, Canada). Plate count agar (OXOID, Dehydrated, OXCM0325B) was purchased from Thermo Scientific ™ (Montreal, QC, Canada). All the chemicals were used without any further purification. Fresh Beef and red cabbage were provided from a local butchery and grocery store in Montreal, Canada.

3.3 Preparation of PLA nanofibrous mat through Electrospinning

PLA granules were solved in TFE under stirring over night at room temperature (23 ºC) and the concentration of solution adjusted to be 15% w/v. Then, PLA solution was filled into a plastic syringe of 5 mL and electrospun at a feed rated of 1 mL/h using a 26-gauge stainless steel needle which was attached to the positive side of a high voltage power supply (ES60P-5W, Ormond Beach, FL, USA). The electrospinning solution was fed to the needle by means of a force applied from an infusion pump (Harvard Apparatus, USA). When the electrostatic force reached the critical value at voltage of 20 kV, the droplet of solution at the needle tip started to stretch that caused the PLA solution to eject in the form of fibers towards the grounded collector plate positioned at 20 cm from the needle tip. The solvent evaporated rapidly during the polymer jet flight in the air. Therefore, solidified fibers are randomly fabricated on the collector plate. The electrospun fibers were collected on an aluminum foil which was attached on the surface of the collector. The

electrospinning procedure were carried out at room temperature (21 ±2 ºC) and relative humidity of 50±5%. Table 3.1 below describes the electrospinning specification.

Table 3.1 Electrospinning specification

Parameter	Specification
Solution concentration	15% w/v
Flow rate	1 mL/h
Voltage	20 kV
Spinneret tip	21-26 gauges
Tip-to-collector distance	20 cm
Humidity	50-55%
Temperature	21-23˚c

3.4 Extraction of natural dye

Red cabbage leaves were washed with H_2O, dried for 24h and cut into small pieces. Extraction was accomplished by taking 150 gr of red cabbage and 80 mL of ethanol while its pH was adjusted to 2 by adding 800 µL of HCL 1M. The mixture was then mixed well in a magnetic stirrer (Corning Inc., USA) for 2h. The extraction media was kept in dark place for 24h at 4℃. The mixture was filtered though a vacuum filter in order to separate the extracted anthocyanin from the red cabbage leaves. Finally, the filtered solution was centrifuged (Thermo Scientific™ Sorvall™ RC 6 Plus) at 10000 rpm for 15 minutes in order to remove the fine particles.

3.5 Preparation of Colorimetric Sensor Platform

PLA electrospun nanofiber mats were immersed into the 20mL anthocyanin solution extracted from red cabbage and then retained for 24 h at room temperature and under a gentile shaking in a lab scale shaker (Standard Analog 1000 Orbital Shaker, 120V, TALBOYS). After, the immersed mats were taken out from the dye solution and positioned on a handmade clotheshorse for 48 h in order to evaporate the residual solvent on the mats.

The drying step was performed at room temperature under the laboratory fume hood to ensure that all of the solvents evaporated.

Colorimetric platform was prepared with three different concentrations of the dye solution, which will be further discussed in next chapter all these concentrations were carried out with pure EtOH.

3.6 Volatile compound sensing and meat spoilage

Sealable containers with total volume of 1000 mL were chosen in order to carry out the volatile compound sensing experiments. To evaluate the sensitivity and selectivity of our gas sensing platform, microscopic droplets of VOCs varies from 5 to 100 µL were drop-casted onto the bottom of the container. The studied compound automatically volatilized and exposed to the sensor platform which was positioned at the wall of the sealed container. The sensor platform (circular shape with a 6mm diameter) was assembled inside of an aluminum foil holder which was horizontally attached to the container. The detection setup was tightly fixed on the scanner (Epson Canada Ltd, Perfection V550) and continuedly be scanned as it is shown in Figure 3.1. All the experiments were carried out at room temperature.

In terms of meat spoilage, our developed sensor platform was applied for fresh beef samples at 100gr each and deposited into the plastic tray which was fixed at the corner of a sealable container. The volatile compound is produced as a result of meat deterioration by microorganisms. Our developed sensor was implanted horizontally to the inside of a sealable container. In order to study the colorimetric detection of the applied sensor the meat spoilage setup was assembled on the scanner as presented in Figure 3.2. Subsequently, meat spoilage experiments were carried out at different temperatures (4 ºC & 23 ºC).

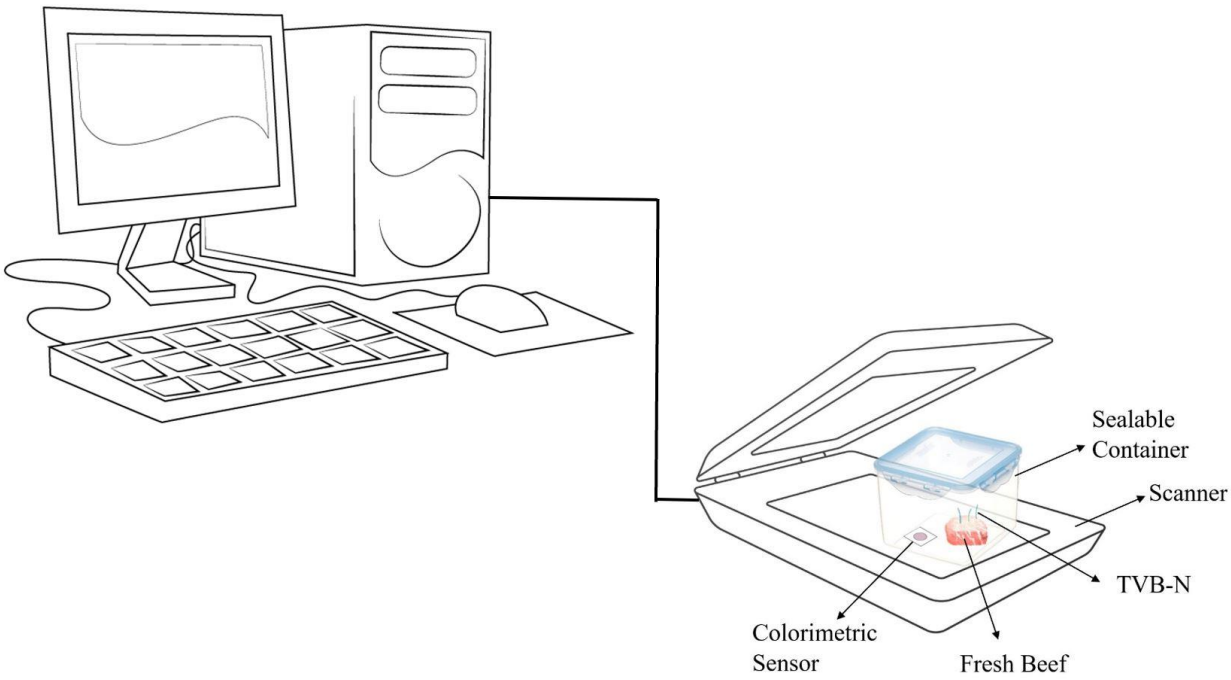


Figure 3.1 Detection of Beef spoilage setup

3.7 Microbial test

Prior to use, all solutions and equipment for the microbiological tests were prepared and sterilised in an autoclave. 120 gr Plate agar count agar plates was dissolved in 100 mL deionized water in order to prepare the bacterial growth medium. The solution was sterilised in an autoclave, before being poured into standard Petri-dish plates. The agar plates were stored in the fridge at 4˚C prior being used.

The beef was cut into 100gr pieces which were placed into the individual plastic trays and sealed with PET plastic lids. After that, 25mL of phosphate buffered saline (PBS, 0.01M) was added to the beef samples and thoroughly mixed with a vortex (Scientific Industries, Vortex Genie 2, Biotech Inc, Montreal Canada) for 5min. 100µL of the obtained solution was serially diluted down to six times (from 10^0 to 10^{-6}). In the final step, three droplets (volume of 1µL) from each dilution were spread onto the surface of dried agar plate to determine the total viable count (TVC) of the

grown bacteria. Agar plates including mediums were incubated (Economy Incubators IB-11E,150L, Biotech Inc, Montreal Canada) at 37℃ for 48 h before counting the colony growth [119].

CHAPTER 4 ARTICLE 1: DEVELOPMENT OF A SENSITIVE COLORIMETRIC SENSOR FOR DETECTING BEEF SPOILAGE IN SMART PACKAGING

Dariush Karimi Alavijeh, Bentolhoda Heli, Abdellah Ajji*

[a]Département de génie chimique, Polytechnique Montréal, Montréal, Québec, H3C3A7, Canada

Article submitted to ACS Applied Materials & Interfaces

Abstract

This work is aimed to develop and characterize a noble colorimetric sensor for total volatile basic nitrogen (TVB-N) and monitoring the freshness of meat. The sensing platform was fabricated from poly lactic acid (PLA) electrospun nanofibers doped with a sensitive natural pH dye, anthocyanins, extracted from red cabbage. The relationship between the concentration of anthocyanins and the color response efficiency of colorimetric platform was investigated. The SEM results showed the morphology and structure of the nanofiber mats before and after the dip coating method. Next, the selectivity of sensor using a variety of volatile organic compounds (VOCs) was explored. Then, the sensitivity of sensor was studied upon exposure to ammonia (NH3) with various concentration from 1-100 ppm. The sensor was confirmed to have a detection limit of 1 ppm when detected by the naked eye. Additionally, the sensor responded accurately to the freshness of the beef, in terms of its pH change due to its deterioration by both color change at room and fridge temperatures. As well as other parameters such as (TVB-N) and total viable count (TVC). Spoilage level was reached after 10 h and 7 days due to the bacteria growth (107 CFU/mL) as the threshold of spoilage at room and fridge temperatures respectively. The stability of the sensor in relation to humidity was also investigated. In conclusion, it was found that the sensor has a great potential to be used as a simple and safe indicator for monitoring packaged beef.

Keywords:

Colorimetric detection, Gas sensor, anthocyanins, freshness indicator, natural dye, meat spoilage, intelligent packaging

4.1 Introduction:

Food safety is one of the major concerns of international organizations due to its macroeconomic and environmental effects. Also, the demand for real time, quality monitoring in food packaging is increasingly advocated by consumers and manufacturers. Freshness is an essential factor for many kinds of protein-based products. Freshness can be described using some sensory characteristics, microbial, chemical and physical parameters [1]. The consumption of spoiled food may not cause illness but, changes in texture and smell often cause them to be rejected by consumers. This food loss causes a considerable effect on economy. According to the United States' Department of Agriculture (USDA), over 133 billion pounds of food in the United States was lost by retailers, foodservice and consumers in 2010 [2].

Recently, smart/intelligent packaging has opened the possibility of monitoring the freshness of food products though the detection of analytes. An intelligent packaging is a system of sensing, detecting and recording any deterioration inside a food package. Many smart packaging techniques are based on sensor technologies that have been researched during past few decades [3-6].

Sensory evaluation is the most important method for monitoring the freshness of meat products. Other approaches such as the microbial technique (TVC), physical measurements (e.g., texture and color) and chemical methods such as total volatile basic nitrogen (TVB-N) and lipid oxidation have been reviewed by Olafsdottir et al. [7]. Microorganisms are the major cause of spoilage in protein-based products. A mixture of volatile compounds such as trimethylamine (TMA), dimethylamine (DMA) and ammonia (NH3), which are known collectively as TVB-N, are produced during the microbial degradation of meat and are considered as he main index of identifying the freshness of meat. Generally, the TVB-N level increases due to the formation of volatile amines during the deterioration of meat such as beef.

Consequently, in recent years, there is an increasing interest in the development of a colorimetric sensing platform for identifying spoilage in meat products as they are small, user friendly, highly sensitive and selective, and are cheap to produce. [8]. Normally colorimetric sensors contain two main parts; the detector agent such as a pH sensitive dye or a plasmonic nanoparticle and a solid substrate support such as a polymeric film, nanofiber mats, filter paper or glass, etc. [9].

Acid or base dyes (pH indicator dye) are halochromic chemical compounds, which are sensitive to the concentration of hydrogen ions. The pH sensitive dyes obtained from natural sources have various advantages due to their low toxicity. Some pH sensitive dyes that come from natural sources such as curcumin and anthocyanin have been used in colorimetric detector applications.

Anthocyanins are a group of phenolic compounds which can be extracted from many sources such as red cabbage, grape skin, berries, etc. The color changes in this pigment are due to the cyanidin, delphinidin, pelargonidin, peonidin and petunidin which are subjected to structural changes in the variation of pH. Some studies introduced the usage of anthocyanin extracted from different natural sources as a colorimetric indicator [8]. Anthocyanin extracted from red cabbage (brassica oleraceae) changes color over a very broad range of pH from red at low pH to blue and green at high pH. Other sources of anthocyanin such as grape skin, provides only a narrow color change at pH<4. In addition, the cost of red cabbage being low in comparison to other sources of anthocyanin makes it attractive for use as a colorimetric sensor [10].

A solid substrate is a second part of sensor that plays an important role on the sensitivity of fabricated sensor. Dye immobilization on a solid substrate consists of three major methods; dip coating [11], drop casting [12] and doping [13, 14], Various types of polymeric and bio polymeric materials have been used as substrates in the forms of film, sol-gel nano porous, and electrospun nanofiber mats such as chitosan, polyethylene terephthalate, poly vinyl alcohol, methyl cellulose, corn starch, poly vinyl alcohol (PVA)/chitosan blends (bio composites) and bacterial cellulose [8, 15-20]. Of all these, the nano porous platform fabricated with electrospinning has generated lots of attention due to the high surface-area-to-volume ratio [21]. The process of electrospinning is the fabrication of nanoscale fibers based on using electrical forces on droplets of polymer solution. A typical electrospinning machine consists of a high voltage source, syringe pump, stainless steel spinneret and collector plate (Figure 4.1). The main advantages of the electrospinning method are its easy use, fabrication of a platform with high surface area and the low cost of processing [22].

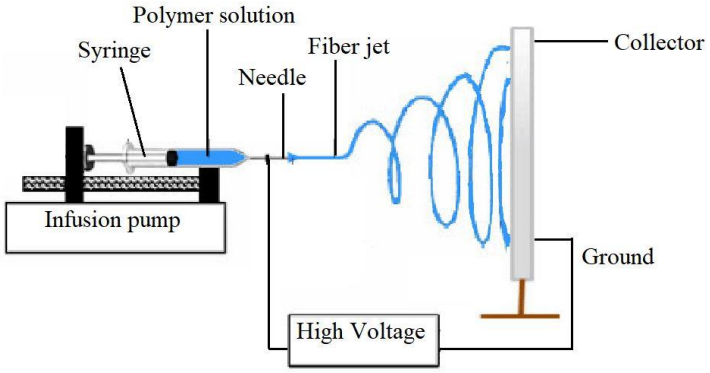


Figure 4-1 Schematic of electrospinning

In this work, our colorimetric sensor shows a high sensitivity and selectivity toward TVB-N (purplish blue coloration detectable to the naked eye even at 1 ppm). This approach demonstrates, with a combination of electrospun substrate imbanded with a naturally sourced, pH sensitive dye that it is an excellent choice for TVB-N detectors listed in Table 4.1.

Table 4.1 List of colorimetric sensors developed with natural dye

Detector agent	Substrate	Application	Ref:
curcumin	k-carrageenan film	pork and shrimp	[23]
curcumin	gum/polyvinyl alcohol film	shrimp	[24]
anthocyanins	Filter paper	shrimp	[25]
anthocyanins	Chitosan film	fish and pork	[8]
anthocyanins	chitosan/PVA films	time-temperature indicator	[17]
anthocyanins	Bacterial-cellulose nanofibers	pH indicator	[9]

anthocyanins	Chitosan film	pH indicator	[26]
anthocyanins	Zein Electrospun mat	pH indicator	[27]
anthocyanins	cassava starch	chilled pork	[28]
anthocyanins	Chitosan film	pH indicator	[16]
anthocyanins	sol–gel film	pH indicator	[29]
anthocyanins	cassava starch film	pH indicator	[30]
anthocyanins	chitosan film	time-temperature indicator	[31]

4.2 Materials and method

4.2.1 Materials

Poly lactic acid (PLA) with the molecular weight of 133,000 gr/mol (INGEOTM Biopolymer 4032D) was supplied from Nature Works LLC (Blair, Nebraska, USA). 2,2,2-Trifluoroethanol (TFE, ≥99.9%), ethanol (EtOH, ≥99.9%), ammonium hydroxide (ACS reagent, 28.0-30.0% NH3 basis), Dimethylamine (DMA, ≥99%), Trimethylamine (TMA, ≥99%) and plate count agar (OXOID, Dehydrated, OXCM0325B) was purchased from Thermo Scientific TM (Montreal, QC, Canada) were purchased from sigma Aldrich, Canada. Red cabbage and Beef (overlong stitch) were purchase from Marché Fu Tai (Montréal, QC, Canada). All chemicals were received and used without any further purification. Bacterial cellulose was provided from Nanonovin Polymer Co. (Mazandaran, Iran).

4.2.2 Preparing the Electrospinning solution

PLA solution with a concentration of 15% (w/v) was prepared as follows; first, the PLA pellets dried at 70 ºC for 4 h in a vacuum. Next, 1.5 g of PLA pellets were dropped into 10 ml of TFE and mixed overnight under stirring at room temperature (23 ºC).

4.2.3 Extraction of Anthocyanin

The extraction was proceeded according to the Fuleki & Francis (1968) procedure with some modifications [32]. Red cabbage leaves were washed and cut into small pieces. The extraction was carried out by adding 150 gr of chopped leaves to 80 ml of ethanol with pH adjusted to 2 using HCL 1 M in Erlenmeyer flask (100 ml) and placed in the fridge (4 ºC) for 24 h. It has been reported that the stability of anthocyanin increases with a decrease in pH level [33]. The anthocyanin extracted from red cabbage was filtered though filter paper (brand filter paper) to remove the red cabbage leaves. The extraction obtained from filtration was centrifuged (Thermo Scientific™ Sorvall™ RC 6 Plus) in 10000 rpm for 15 minutes to remove fine suspended particles and finally the pH of obtained filtrate was adjusted to be 2 using HCL 1 M. The concentration of the anthocyanins was calculated by pH differential method using following equation [33]:

Pigment concentration (mg/mL) $C = \frac{A \times \mathrm{MW} \times D_f}{\varepsilon} \times L$ Equation 4.1

Whereas A represents (A530 - A700) pH 1.0 – (A530 – A700) pH 4.5, MW is the molecular weight of anthocyanin (449 g/mol), Df represents the dilution factor, ε is the extinction coefficient (26,900 L/cm2 mol) and L is the path length (1cm).

4.2.4 Fabrication of the Colorimetric Electrospun PLA Nanofiber Platform

To obtain the electrospun nanofiber platform, the PLA solution was filled in a 5 ml plastic syringe, connected to a 23-gauge, stainless steel needle. Then, it was placed in a syringe pump (Harvard Apparatus, PHD2000, USA) and the positive electrode of a power supply was clipped to the needle. . The polymer solution was electrospun at a feed rate of 1 mL/h with the tip-to-collector distance of 15cm. Through applying a voltage of 20 kV provided by the high-voltage power supply (ES60P-5W Gamma high voltage research Inc, Ormond Beach, FL, USA) a nonwoven nanofiber were fabricated on a grounded stationary collector, covered with aluminum foil. All the

electrospinning experiments were accomplished at room temperature (23 ºC) and a relative humidity around 50%.

Anthocyanin extracted from red cabbage was immobilized on a PLA electrospun nanofiber and bacterial cellulose using the dip coating method. In brief, a central piece of PLA substrate cut in the size of 5×5 cm and the average thickness 0.4 mm, immersed into 20 ml of the dye solution and placed on shaker (Standard Analog 1000 Orbital Shaker, 120V, TALBOYS) for 24 h. In order to optimize the concentration of anthocyanin for the sensing platform, dye solutions with various concentrations were prepared (the initial solution of extracted dye was diluted 10 and 20 times by ethanol). The immobilization of the dyes is attributed to the physical adsorption between the nano porous platforms and the anthocyanin pigments. In addition, the sensitivity of nanofibrous electrospun-based colorimetric sensor platform was compared with those of the bacterial cellulose-based substrates. As such, the bacterial cellulose substrate cut in the size of 5×5 cm and the average thickness 0.4 mm, immersed into 20 ml of the dye solution and placed on shaker for 24 h. the drying steps were carried out by first, positioned the mats on a handmade clotheshorse for 48 h in order to evaporate the residual solvent on the mats then, placed under the laboratory fume hood at room temperature to ensure that all of the solvents evaporated.

4.2.5 Characterization of the Electrospun Nanofiber Mats

The morphology of the resulted electrospun nanofibers was analyzed using a scanning electron microscope (SEM, TM3030PLUS, HITACHI) operating at 10-15 kV. The fiber diameter was also evaluated using open source LAB ORIGIN software. The weight and thickness of the sheets were measured using a balance (ML 3002E, Mettler Toledo, Switzerland) and a micrometer (Mitutoyo 547-401 ABSOLUTE Digimatic Thickness Gauge), respectively. The porosity of the nanofibrous mats were calculated using two different methods: apparent density measurements and liquid (ethanol) intrusion [34]. For the apparent density method, the density of electrospun mat (ρ) was calculated from obtaining the weight and thickness with a circular area of 6 mm diameter which was precisely punched. By knowing the density (ρ0) value of1.3 g/cm-3 and 1.25 g / cm-3 for both nano porous mats (bacterial cellulose and PLA, respectively) [35], the porosity (P%) was calculated with following equation:

$$P\% = \frac{\rho_0 - \rho}{\rho_0} \times 100$$ Equation 4.2

Porosity of mats were also determined using a liquid (ethanol) intrusion method [34]. In such a method, dry mats with the size of 5×5 cm were weighted and immersed in pure EtOH overnight. Mats were then gently wipe in order to remove surplus EtOH and weight again. Porosity is defined as the volume of EtOH entrapped in the pores divided by the total volume of wet mats.

4.2.6 Evaluating the Performance of the Colorimetric Platform by exposing to ammonia

The colorimetric gas sensing experiments were done using a homemade detection setup comprising of a sealable glass container with a total volume of 900 mL placed on a scanner (Epson Canada Ltd, Perfection V550).

The developed sensor was exposed to the evaporated NH3 at different concentrations varying from 1 to 100 ppm, that was provided through the simultaneous evaporation of an ammonia solution with various concentrations at room temperature the concentration of NH3 was calculated using the following equation:

$$NH3\ concentration = \frac{M \times T}{V \times Mw \times 12.178}$$ Equation 4.3

Where M represents the weight of NH3 (mg), T is temperature (K), V represents the volume of container (900 ml) and MW is the molecular weight of NH3 (17 g/mol).

Subsequently, digital images were captured by scanner during the exposure of NH3 in a period of 60 minutes by scanning every simultaneously under a darkened surrounding area.

In order to verify the selectivity and specificity of the developed colorimetric sensor, the volatile organic compounds such as Dimethylformamide, X-xylene, Formaldehyde, Ethanol, Tetrahydrofuran, Methanol, Dichloromethane, Acetone and volatile based nitrogen such as Dimethylamine, Trimethylamine, Ammonium hydroxide were exposed into the sensors. As such,100 µL of the specific solution was drop casted into the sealed container and the vapor was

supplied by the evaporation at room temperature. The color change of the sensor was recorded as mentioned before.

The evaluation of the colorimetric responses was carried out under the room temperature and relative humidity of 100%. All the experiments were carried out for PLA nanofiber mats and bacterial cellulose sheets in order to study the effect of a different substrate on the performance of the colorimetric sensor.

The scanned image of the initial and exposed sensing platforms was imported into MATLAB (version 9.5.0, Copyright (c) 2018, Polytechnique Montreal) for quantitative analysis. The RGB difference model (ΔRGB) was used to read the color information and differences before and after exposure to NH3 and also other compounds. The RGB model in MATLAB decomposes the color of each image into thee components of red (R), green (G) and blue (B) and quantitively shows them in a three-dimensional matrix. By calculating the difference of these components in each image, the performance of the sensing platform can be evaluated. The following equation represents the calculation of this model where the subscript 0 corresponds to the initial image.

$$\Delta RGB=\sqrt{(R-R_0)^2+(G-G_0)^2+(B-B_0)^2} \quad \text{Equation 4.4}$$

4.2.7 Sensitivity of the colorimetric sensor for detecting the Meat spoilage

As a proof-of-concept, the developed sensor was applied to examine the freshness of a real packed meat through its sensitivity toward the produced gases during the spoilage of a meat. This sensitivity was also validated by comparing with the microbial growth within meat samples. As such, 100 gr of fresh beef was cut and prepared under sterile conditions and then transferred to the sealable container with the sensor (circular shape with 6 mm diameter assembled inside aluminum foil), which, was the horizontally attached to the sealed container. The whole sensing setup was assembled onto the scanner which was plugged into the computer system to simultaneously scan and process the sensing behavior of the colorimetric sensor.

4.2.7.1 Bacterial analysis

To analysis the presence of bacteria on a piece of meat, one hundred grams of fresh beef, cut under sterile conditions, was mixed with 25 mL of PBS (0.01 M, pH 7.4). Then, the mixture was shaken

and homogenized on a vortex (Scientific Industries, Vortex Genie 2, Biotech Inc, Montréal Canada) for 5 min. Next, 0.1 mL of the prepared sample was serially diluted down to six times. Finally, 0.01 mL of each dilution was spread onto the surface of agar plate in order to determine the TVC of bacterial growth. The agar plate was kept in the incubator (Economy Incubators IB-11E, 150 L, Biotech Inc, Montréal, Canada) for 48 h at 37 ºC in order to grow the bacterial colonies prior to counting [36].

4.3 Result and discussion

4.3.1 Characterization of nanofiber mats

The SEM images of the electrospun PLA and bacterial cellulose are illustrated in Figure 4.2 which displays a morphology of neat mats consisting of randomly oriented nanofibers. The average diameter of 0.9±0.15 µm was observed from the analysis of the nanofibers before dip coating (Figure 4.2 a,c). The morphology of the nanofibrous mats were maintained after the dye coating (Figure 4.2 b,d) without any significant changes on the average diameter of the nanofibers. However, the porosity of the dye-impregnated nanofiber samples displayed a slight decrease due to the increase in the weight of the mats after the coating. The properties of the prepared platforms through both substrates are thoroughly compared in Table 4.2. Therefore, the coating process did not have a significant effect on the properties of nanofibrous mats. This result shows that the homogeneously anthocyanin-impregnated nano porous layer can be prepared by the simple dip-coating process.

According to the results, measured porosity of mats by mentioned methods have shown the similar results.

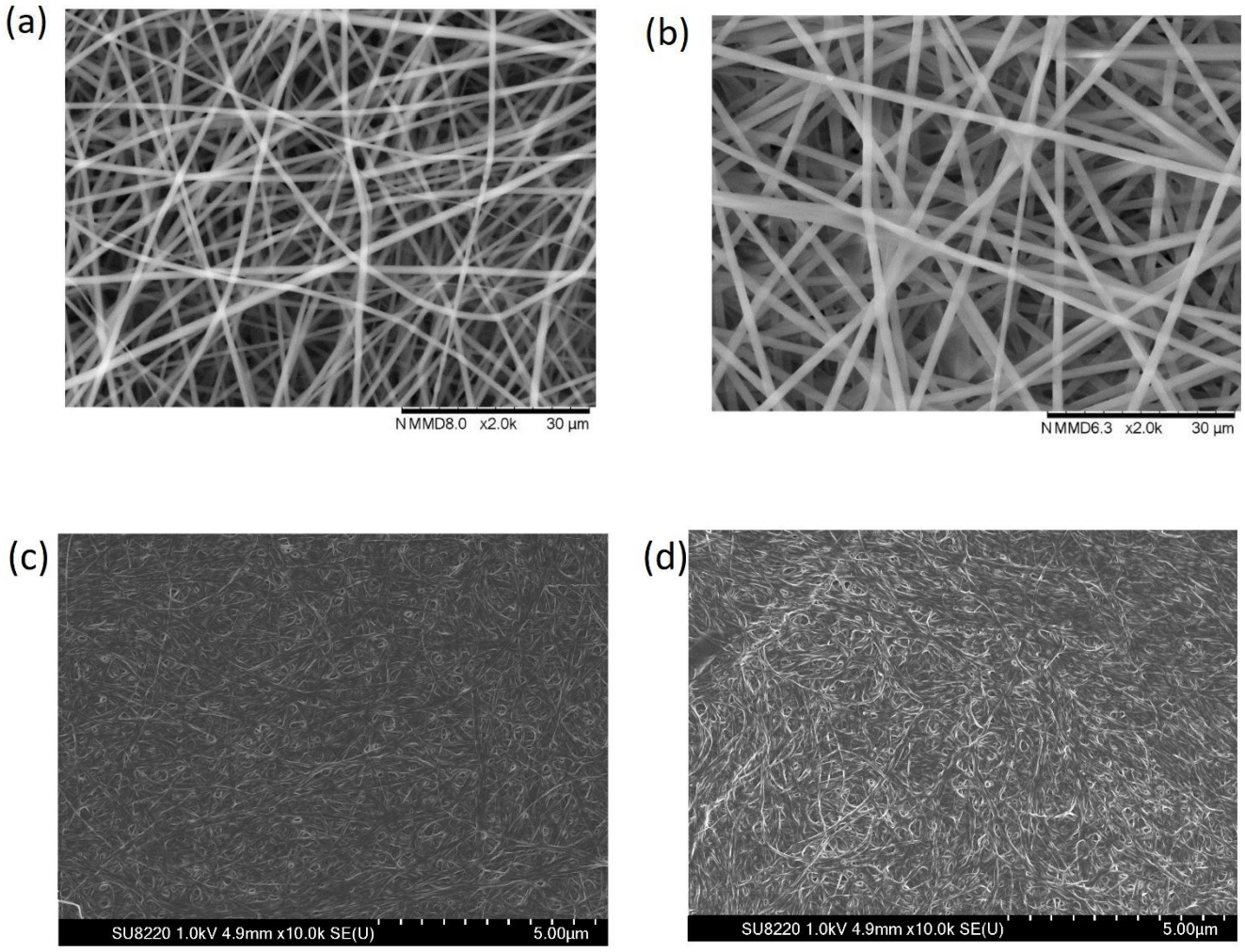


Figure 4-2 SEM images of a. and c. is Electrospun PLA nanofibers and bacterial cellulose, b. and d. are the anthocyanin-impregnated samples

Sample	Diameter (µm)	Thickness (µm)	Density(gr/cm^3)	Porosity %	Liquid intrusion method
PLA nanofiber neat sample	0.9 ±0.15	400 ±56	0.175	86 ± 1	87 ± 2

Dye-impregnated PLA nanofiber sample	1.02±0.15	400 ±70	0.183	84 ± 2	84 ± 3
BC	0.33±0.08	20 ±10	1.036	21 ± 1	23 ± 2
dye-impregnated BC	0.36±0.05	20±5	1.04	20 ± 2	21 ± 2

Table 4.2 Comparison the properties of two different sensor platforms.

4.3.2 Selectivity of the sensor through different VOCs

The selectivity of the proposed sensor was evaluated by measuring the ΔRGB in the presence of various VOCs with the defined concentration. As shown in Figure 4.3, a significant change in color was observed when the sensor was exposed to the DMA, TMA and NH3, changing from pink to violet, pink to green and pink to blue respectively. By contrast, the color of sensor remained unchanged when it was exposed to the other organic compounds.

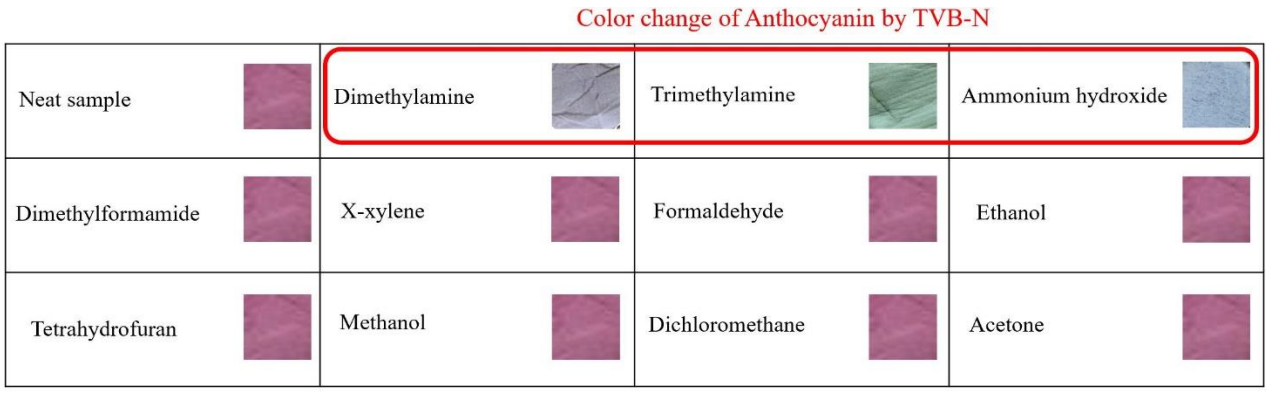


Figure 4-3 Selectivity of the sensor within the VOCs

4.3.3 Examining the sensitivity of the colorimetric sensor toward ammonia

First, different concentrations of the extracted dye were prepared and tested in order to discover the optimal condition of anthocyanin as a pH indicator dye in terms of sensitivity and colorimetry. The sensitivity and colorimetric behavior of sensor were investigated while exposure to NH3 at different concentrations varying from 1 to 100 ppm. The initial concentration of anthocyanin pigment (0.57 mg/mL) in the extracted solution was diluted 10 and 100 times. Each dilution was performed by adding EtOH to the initial concentration of the extracted dye. Figure 4A shows the images taken after exposure of NH3 (60 min) at different concentrations within a range of 1 and 100 ppm.

All the sensors show the purple color with a different intensity at an unexposed state. When the NH3 is exposed, the dye molecules become deprotonated by an acid-base reaction and their initial color gradually changes to blue. By decreasing the concentration of dye, the reactive site of the sensor is diminished. Accordingly, a lower concentration of NH3 gas is needed to react with dye. Therefore, by diluting the dye, the sensitivity is improved as shown in Figure 4.4. However, by decreasing the concentration of the dye in sensor, the RGB value descends and it becomes difficult to distinguish the color change with the naked eye (Figure 4.4 A). The RGB analysis is carried out because of the detection limit of the human eye which is semi-quantitative and varies in terms of color detection. The colorimetric response (ΔRGB) as a function of the NH3 concentration for the sensor impregnated with different dye concentration after NH3 exposure of 60 minutes was investigated and reported in Figure 4.4 B. The calibration curve for NH3 detection presented for the various dye concentration, exhibits a great variation in the range of 1 to 100 ppm. For the dye concentration of 0.57 (mg/mL) (Figure 4.4 (a)), the saturation plateau occurred at 30 ppm while for dye concentration of 0.057 (mg/mL) (Figure 4.4 (b)) and 0.0057 (mg/mL) (Figure 4.4 (c)), calibration curves reached the plateau in the region of 1ppm, as recognized by the naked eye. Since the slop of the curves represent the sensitivity of sensors, the logarithmic increase demonstrates a more effective detection at a lower NH3 concentration. Hence, by modification of dye solution, the limit of detection be improved to lower 1 ppm. However, the anthocyanin concentration of 0.0057(mg/mL) may not be applicable for the sensor.

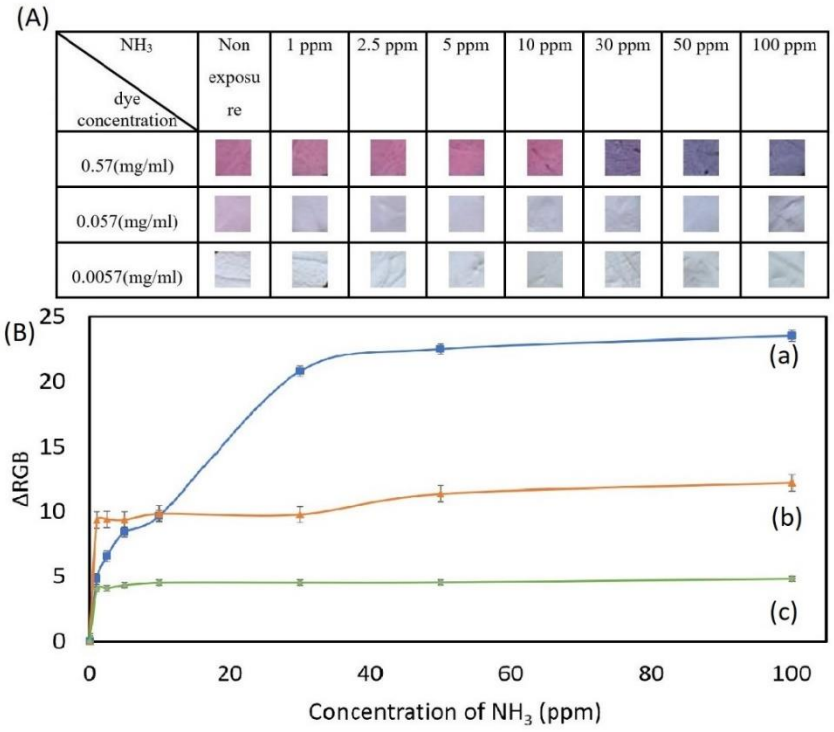


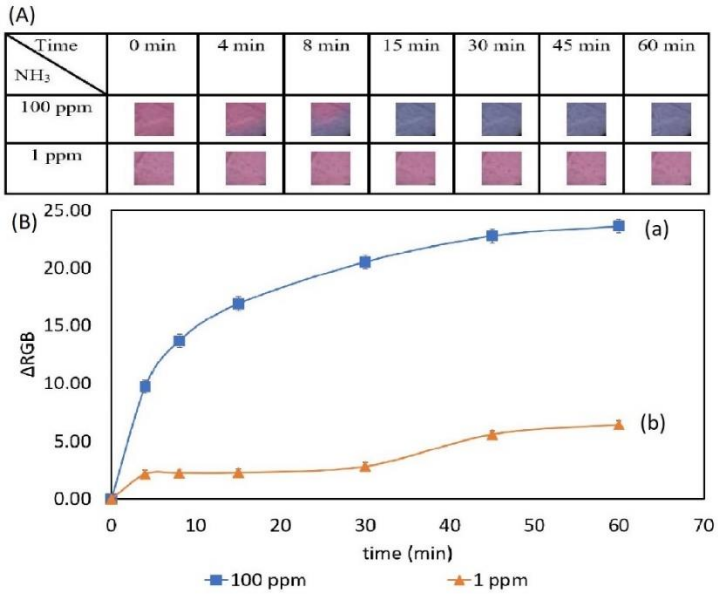
Figure 4-4 (A) Optical image of sensor captured at different NH_3 concentration in the range of 1-100 ppm and (B) The corresponding calibration curve of the concentration and ΔRGB for dye concentration of (a). 0.57 (mg/mL), (b). 0.057 (mg/mL) and (c). 0.0057 (mg/mL)

A study of the kinetic reaction was carried out in order to investigate the color changing behavior of anthocyanin impregnated in the PLA nanofiber sensor. Figure 4-5 B, D, F shows the RGB distance (ΔRGB) as a function of time with respect to the dye concentration. The optical images of the sensors at different times during 60 minutes exposure of NH3 represent the variation of color from pink to blue during the exposure time. Figure 4-5 A. shows the color change for the dye concentration of 0.57 (mg/mL) for 60 minutes exposure of 100ppm and 1ppm of NH3. the pink color changed and saturated to blue within 15 minutes during the 100ppm NH3 exposure, meanwhile, when it was 1 ppm, the sensor did not exhibit a significant change in color. However, as shown in Figure 4.5 C and E, for dye concentration of 0.057 (mg/mL) and 0.0057 (mg/mL) the initial pink color changed and saturated to blue within the first minute when exposed to 100 ppm NH3 but upon exposure to 1 ppm, the pink color slowly approached a blue color for the 30 minutes

of NH3 supply. However, color change for the dye concentration of 0.0057 (mg/mL) is not significant and the RGB distance is in the range of error for the naked eye.

The slow response indicates that the ΔRGB kinetics is limited by the transfer rate of NH3 molecules at low concentration. In addition Hoang et al. reported that the kinetic color change of the sensor is limited by mass transfer [37].

The blue color first appears at the edge of sensors and then propagates to the whole surface of the sensors, which is probably due to the direction of flow of NH3.



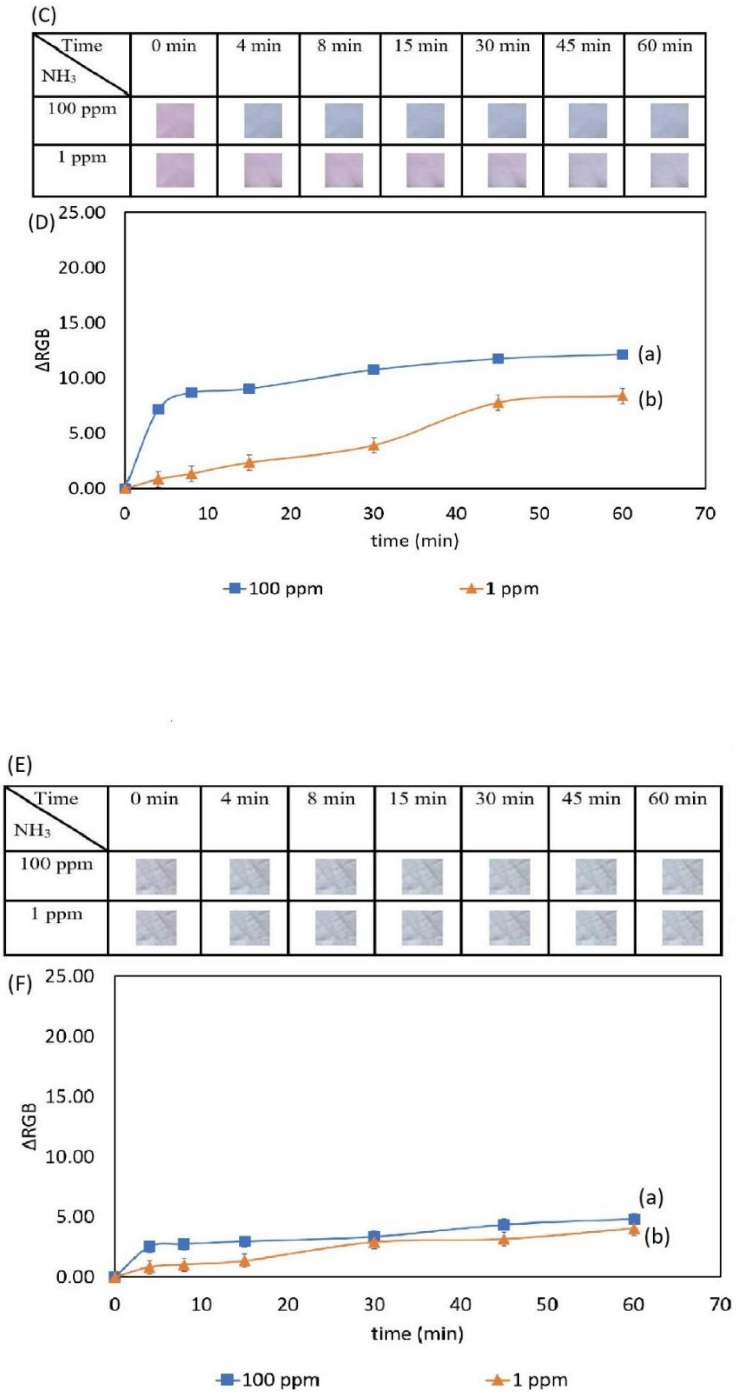


Figure 4-5 (B),(D) and (F) show the variation of the RGB distance at the concentration of (a)100 ppm and (b) 1 ppm with respect to the dye concentration and (A), (C) and (E) shows the optical images of sensor as a function of detection time after the NH_3 exposures with dye concentration of 0.57 (mg/mL) , 0.057 (mg/mL) and 0.0057 (mg/mL), respective

As reported earlier, to compare the sensitivity of the PLA electrospun nanofiber mats with other substrates, the bacterial cellulose also was applied as a solid matrix support in the sensor. Figure 4-6 A. and B. reveals the results of NH3 detection and comparison of different substrates. As it is clear, PLA porous media represent better results in terms of sensitivity at a higher NH3 concentration (100 ppm), which is probably due to its high porosity and surface area. However, in a lower concentration of NH3 (1 ppm), the colorimetric results are almost at the same range and this is probably because at lower concentration of NH3, the kinetic reaction is independent from porosity. Another limitation of the bacterial cellulose for a colorimetric sensor application is its transparency and visual properties when compared with polymeric electrospun medias.

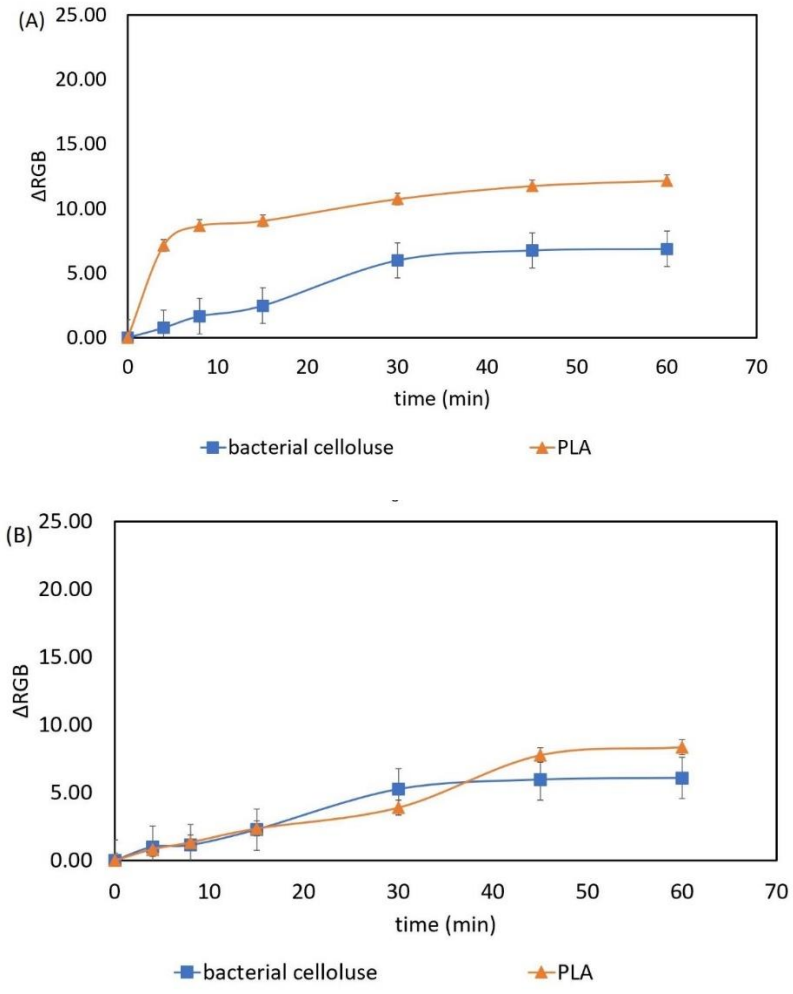
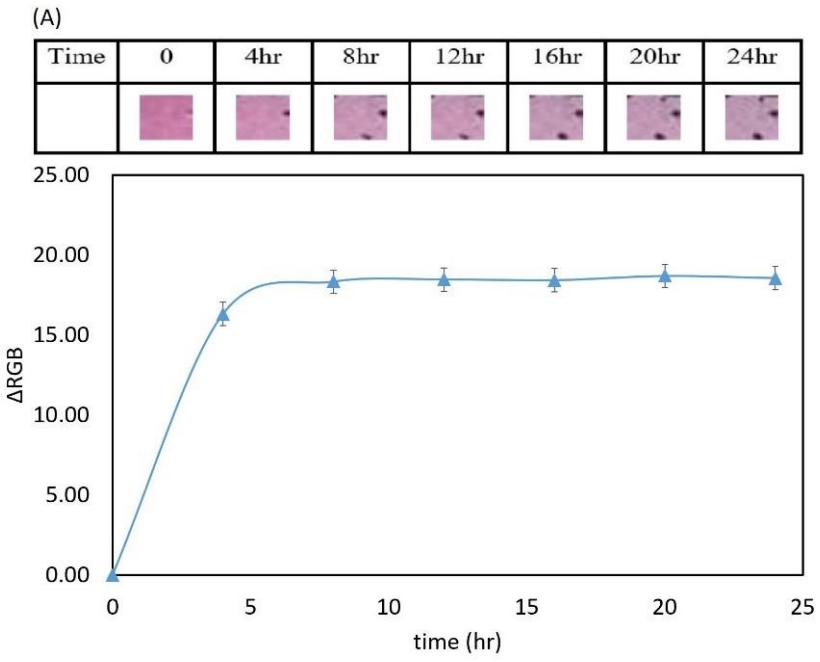


Figure 4-6 RGB distance as a function of NH_3 exposure time (A) 100 ppm (B) 1 ppm for (a) PLA electrospun mats and (b) bacterial cellulose as a substrate

4.3.4 Colorimetric Sensing of Meat spoilage

The detecting range of the developed sensor corresponds to the TVB-N value from 1 to 100 ppm, while the TVB-N threshold of 25mg/100 g is usually reported for fresh meat [38]. Accordingly, based on sensing analysis of the colorimetric platforms (Figure 4.4), the best condition of dye for evaluating the meat spoilage is 0.57 (mg/l), since other concentration of dye reaches to its saturation point at a low concentration of NH3 (1 ppm).

The spoilage of beef was investigated for 24 h at room temperature (23 ºC) and fridge condition (4 ºC) by evaluating the colorimetric sensor response. Figure 4.7 illustrates the RGB analysis and optical images of the sensor after testing with 100 gr of fresh beef (A) during 24 h at room temperature (23 ºC) and (B) during nine days at 4 ºC. The changes in the color of the sensor indicate the presence of TVB-N that are generated during the meat spoilage. As expected, the color changed completely and saturated before spoilage.



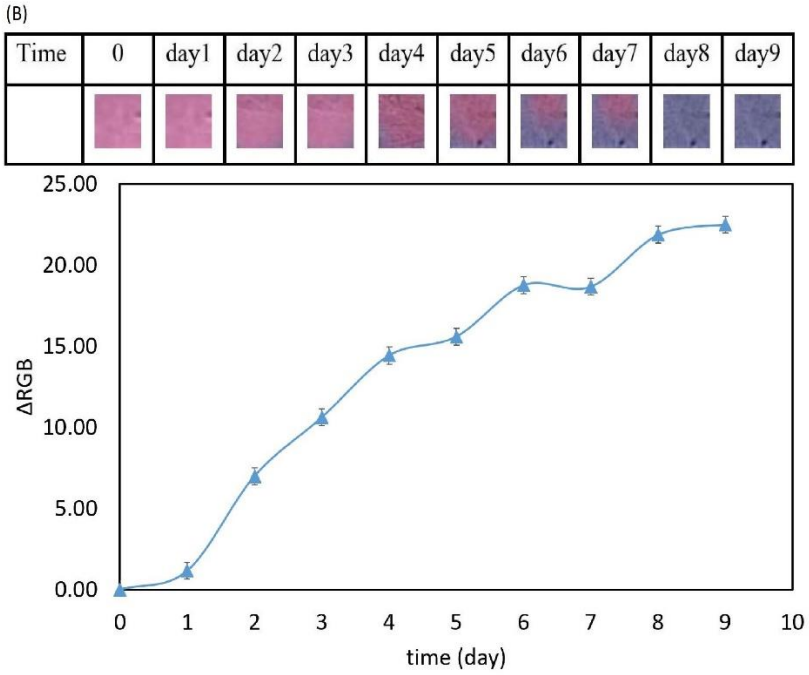


Figure 4-7 Colorimetric analysis and optical image of sensor during the beef spoilage for (A) room temperature (23 ºC) in 24h and (B) for 4 ºC in nine days

4.3.5 Bacteria counting

The bacterial growth was investigated using the TVC method during the storage of meat at room temperature (23 ºC) and fridge condition (4 ºC), indicating that the TVC reached the threshold of bacterial spoilage (1×107 CFU/mL) [39] after 10 h for room temperature (Figure 4.8 (A)) and seven days for fridge condition (Figure 4.8 (B)). For the colorimetric platform, the changes could be detected after 8 h for room temperature and seven days at fridge condition based on the RGB analysis that indicates this result is well fitted and corelated with the bacterial growth.

The senor shows the good sensitivity and the color change that could be detectable before a complete spoilage of meat. While the TVC increases considerably from the threshold point, the color change intensity of the colorimetric sensor reaches the plateau, illustrating a saturation of sensor.

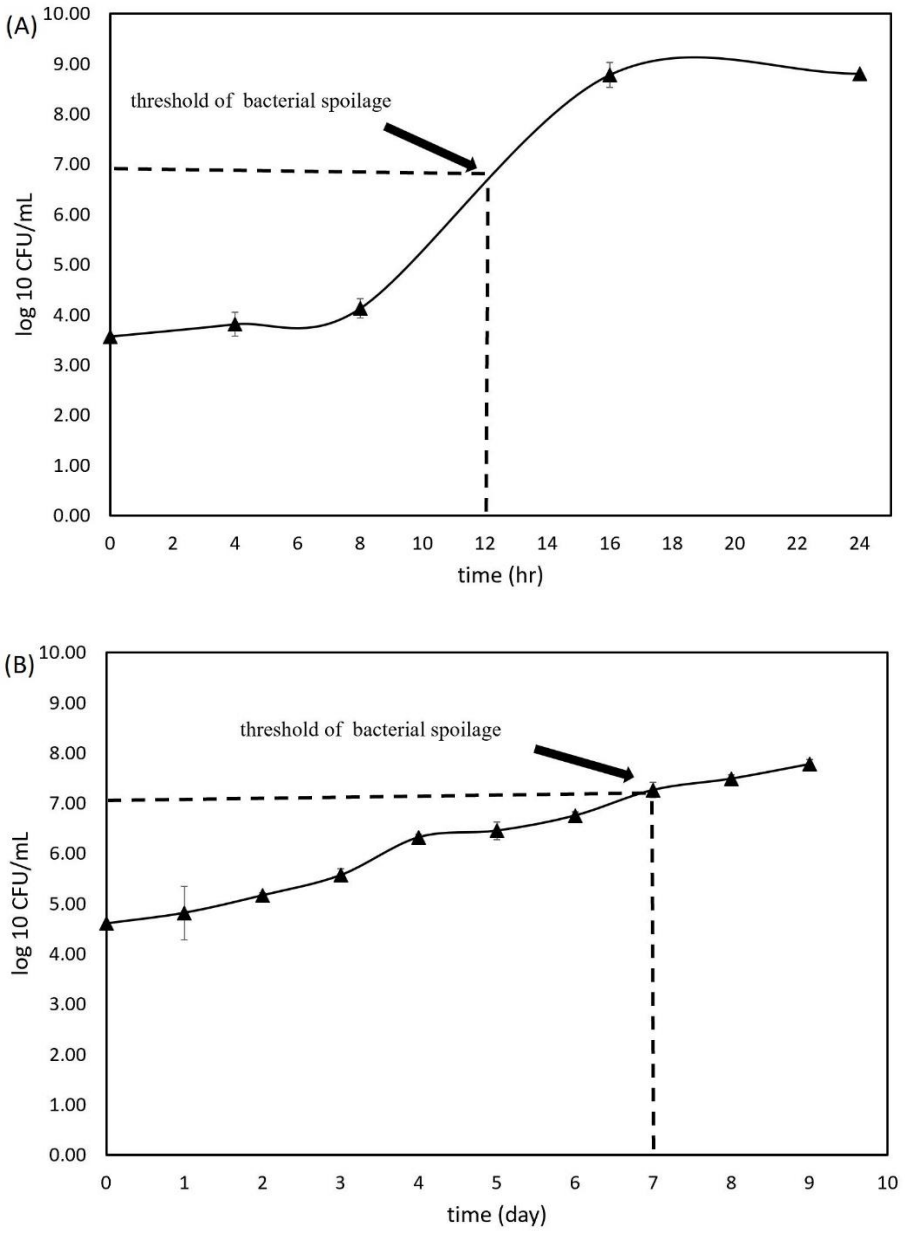


Figure 4-8 TVC count during storage (A) 23 ºC followed up to 24 h and (B) at 4 ºC followed up to nine days

4.3.6 Stability of sensor with humidity

The stability of sensor under moisture is an important characteristic, which can strongly define the applicability of the platform. To determine H2O resistance of the sensor, prepared samples were

exposed to the nitrogen gas with 100% humidity for 12 h and the visual property was studied. Table 4.3 shows the optical image of the sensor before and after exposure to the moisture.

Table 4.3 Optical images of sensor before and after exposure to the moisture

Concentration dye / Time	0.57 (mg/ml)	0.057 (mg/ml)	0.0057 (mg/ml)
0			
6h			

4.4 Conclusion

In conclusion, we reported the fabrication of a novel colorimetric TVB-N sensor consisting of pH sensitive dye from a natural source. The sensing platforms were approved to have admissible stability and high performance in sensitivity. A rapid detection time of less than 1 min for concentrations of 30 ppm, admirable selectivity though TVB-N were observed. Considering the 10 times dilution of anthocyanin extracted from red cabbage, the sensor demonstrates sufficient sensitivity for naked eye detection even with a short exposure time of 1 ppm gaseous NH3. Taking advantage of the acid-base reaction between anthocyanins and dissolved NH3, our presented sensor shows a limit of detection of less than 1ppm in a short period of time. This work has revealed a simple colorimetric platform which has the promising potential for use in different applications especially with food packaging industries in order to display the freshness of products.

4.5 Acknowledgments

The authors would like to acknowledge PROMPAC for their financial support. The authors gratefully acknowledge the support of The Natural Sciences and Engineering Research Council of

Canada (NSERC) and 3SPack Chair Saputo Industries. The authors gratefully appreciate Dr. Davoud Karimi for his remarkable support and encouragement in this project.

1. Howgate, P., et al., Multilingual guide to EC freshness grades for fishery products, December 1992. 1992.
2. Alexander, C., N. Gregson, and Z. Gille, Food waste. The handbook of food research, 2013. 1: p. 471-483.
3. Avella, M., et al., Nanocomposite sensors for food packaging, in Nanotechnological Basis for Advanced Sensors. 2011, Springer. p. 501-510.
4. Mukhopadhyay, S., et al. A Novel Bio-sensor for Non-invasive Meat Inspection. in SENSORS, 2006 IEEE. 2006. IEEE.
5. Abad, E., et al., Flexible tag microlab development: gas sensors integration in RFID flexible tags for food logistic. Sensors and Actuators B: Chemical, 2007. 127(1): p. 2-7.
6. Borchert, N.B., J.P. Kerry, and D.B. Papkovsky, A CO2 sensor based on Pt-porphyrin dye and FRET scheme for food packaging applications. Sensors and Actuators B: Chemical, 2013. 176: p. 157-165.
7. Olafsdottir, G., et al., Multisensor for fish quality determination. Trends in food science & technology, 2004. 15(2): p. 86-93.
8. Zhang, X., S. Lu, and X. Chen, A visual pH sensing film using natural dyes from Bauhinia blakeana Dunn. Sensors and Actuators B: Chemical, 2014. 198: p. 268-273.
9. Pourjavaher, S., et al., Development of a colorimetric pH indicator based on bacterial cellulose nanofibers and red cabbage (Brassica oleraceae) extract. Carbohydrate polymers, 2017. 156: p. 193-201.
10. Chandrasekhar, J., M. Madhusudhan, and K. Raghavarao, Extraction of anthocyanins from red cabbage and purification using adsorption. Food and bioproducts processing, 2012. 90(4): p. 615-623.
11. Wang, X., et al., A facile and highly sensitive colorimetric sensor for the detection of formaldehyde based on electro-spinning/netting nano-fiber/nets. Sensors and Actuators B: Chemical, 2012. 163(1): p. 186-193.
12. Hoang, A.T., Y.B. Cho, and Y.S. Kim, A strip array of colorimetric sensors for visualizing a concentration level of gaseous analytes with basicity. Sensors and Actuators B: Chemical, 2017. 251: p. 1089-1095.

13. Wang, D., et al., Enhanced formaldehyde sensing properties of hollow SnO2 nanofibers by graphene oxide. Sensors and Actuators B: Chemical, 2017. 250: p. 533-542.
14. You, M.-H., et al., Colorimetric humidity sensors based on electrospun polyamide/CoCl 2 nanofibrous membranes. Nanoscale research letters, 2017. 12(1): p. 360.
15. Kanmani, P. and J.-W. Rhim, Antimicrobial and physical-mechanical properties of agar-based films incorporated with grapefruit seed extract. Carbohydrate Polymers, 2014. 102: p. 708-716.
16. Yoshida, C.M., et al., Chitosan biobased and intelligent films: Monitoring pH variations. LWT-Food Science and Technology, 2014. 55(1): p. 83-89.
17. Pereira Jr, V.A., I.N.Q. de Arruda, and R. Stefani, Active chitosan/PVA films with anthocyanins from Brassica oleraceae (Red Cabbage) as time–temperature indicators for application in intelligent food packaging. Food Hydrocolloids, 2015. 43: p. 180-188.
18. Silva-Pereira, M.C., et al., Chitosan/corn starch blend films with extract from Brassica oleraceae (red cabbage) as a visual indicator of fish deterioration. LWT-Food Science and Technology, 2015. 61(1): p. 258-262.
19. Rukchon, C., et al., Development of a food spoilage indicator for monitoring freshness of skinless chicken breast. Talanta, 2014. 130: p. 547-554.
20. Maciel, V.B.V., C.M. Yoshida, and T.T. Franco, Chitosan/pectin polyelectrolyte complex as a pH indicator. Carbohydrate polymers, 2015. 132: p. 537-545.
21. Yoon, J., S.K. Chae, and J.-M. Kim, Colorimetric sensors for volatile organic compounds (VOCs) based on conjugated polymer-embedded electrospun fibers. Journal of the American Chemical Society, 2007. 129(11): p. 3038-3039.
22. Mercante, L.A., et al., Electrospinning-based (bio) sensors for food and agricultural applications: A review. TrAC Trends in Analytical Chemistry, 2017. 91: p. 91-103.
23. Liu, J., et al., Films based on κ-carrageenan incorporated with curcumin for freshness monitoring. Food hydrocolloids, 2018. 83: p. 134-142.
24. Ma, Q., L. Du, and L. Wang, Tara gum/polyvinyl alcohol-based colorimetric NH3 indicator films incorporating curcumin for intelligent packaging. Sensors and Actuators B: Chemical, 2017. 244: p. 759-766.

25. Listyarini, A., W. Sholihah, and C. Imawan. A paper-based Colorimetric Indicator Label using Natural Dye for Monitoring Shrimp Spoilage. in IOP Conference Series: Materials Science and Engineering. 2018. IOP Publishing.
26. Halász, K. and L. Csóka, Black chokeberry (Aronia melanocarpa) pomace extract immobilized in chitosan for colorimetric pH indicator film application. Food packaging and shelf life, 2018. 16: p. 185-193.
27. Prietto, L., et al., Ultrafine fibers of zein and anthocyanins as natural pH indicator. Journal of the Science of Food and Agriculture, 2018. 98(7): p. 2735-2741.
28. Golasz, L.B., J.d. Silva, and S.B.d. Silva, Film with anthocyanins as an indicator of chilled pork deterioration. Food Science and Technology, 2013. 33: p. 155-162.
29. Nguyen, L.T., et al., Biological, chemical, and electronic applications of nanofibers. Macromolecular materials and engineering, 2013. 298(8): p. 822-867.
30. Veiga-Santos, P., C. Ditchfield, and C.C. Tadini, Development and evaluation of a novel pH indicator biodegradable film based on cassava starch. Journal of Applied Polymer Science, 2011. 120(2): p. 1069-1079.
31. Martins, J.T., M.A. Cerqueira, and A.A. Vicente, Influence of α-tocopherol on physicochemical properties of chitosan-based films. Food hydrocolloids, 2012. 27(1): p. 220-227.
32. Fuleki, T. and F. Francis, Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries. Journal of food science, 1968. 33(1): p. 72-77.
33. Fuleki, T. and F. Francis, Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice. Journal of food science, 1968. 33(1): p. 78-83.
34. Savoji, H., et al., Plasma-etching for controlled modification of structural and mechanical properties of electrospun PET scaffolds. Plasma Processes and Polymers, 2015. 12(4): p. 314-327.
35. Yousefi, H., et al., Cellulose nanofiber board. Carbohydrate polymers, 2018. 187: p. 133-139.

36. Ahmed, E. and C. Carlstrom, Food microbiology: A laboratory manual. A John Wiley & sons, Inc. Publications, 2003.
37. Hoang, A.T., et al., Sensitive naked-eye detection of gaseous ammonia based on dye-impregnated nanoporous polyacrylonitrile mats. Sensors and Actuators B: Chemical, 2016. 230: p. 250-259.
38. Chang, L.-Y., et al., One-minute fish freshness evaluation by testing the volatile amine gas with an ultrasensitive porous-electrode-capped organic gas sensor system. ACS sensors, 2017. 2(4): p. 531-539.
39. Pacquit, A., et al., Development of a smart packaging for the monitoring of fish spoilage. Food Chemistry, 2007. 102(2): p. 466-470.

CHAPTER 5 GENERAL DISCUSSION

Ongoing research in the field of freshness sensors in intelligent packaging, reflects the essential demands for the improvement of food safety and decreasing waste[30, 136, 150]. The detection of VOCs has been considered an efficient strategy to define the quality of food products specially meats [134, 136]. During past decades, the stunning advances in the employment of natural-based materials, as well as the discovery and improvements of their marvelous properties, have encouraged and accelerated the fabrication of colorimetric sensors[11, 85, 92, 133, 151]. As studied widely in Chapter 2, a broad range of materials enabled the development of a colorimetric sensor for VOCs.[6, 13, 39-41, 48, 118, 152, 153]. Sensitivity, selectivity, ease of use and non-toxicity are the predominant critical parameters that are considered when designing an appropriate colorimetric sensor. This research was aimed at fabricating a novel, colorimetric sensor, which could satisfy the parameters mentioned above, along with suitable applicability in food packaging.

Based on the literature, most of the halochromic dyes are toxic and not applicable for food packaging. Meanwhile, the dyes obtained from natural sources possess the potential to overcome this issue. Hence, it was found that anthocyanins (extracted from red cabbage) as a pH-sensitive dye, which is well coated onto the PLA electrospun nanofiber mat, owing to the excellent properties such as selectivity and sensitivity. Moreover, it can successfully participate in the detection of TVB-N during the deterioration of meat. However, it was some challenges with the coating procedure. Extracted dye with H_2O and H_2O/EtOH didn't well coat on the mats, but with the EtOH alone it well coated. It is probably due to the H_2O large surface tension.

There was some challenges through the fabrication of colorimetric platform

For example, we have tried different average fiber diameter for PLA nano finer mat by changing the electrospinning parameter such as voltage and PLA concentration in solution. For the average diameter under the 1 µm, we had a problem with coating step. The dye did not disperse well and uniform on the mat with small average fiber diameter. On the other hand, for larger average fiber diameter the same problem occurred

Among various type of compounds which are produced from spoilt meat, NH_3 has been assessed more as a target of colorimetric detection due to the high reactivity with pH dyes[80]. As a proof of concept, we investigated the detection of NH_3 by applying anthocyanin as the detector agent

(discussed in Chapter 4). It is worth mentioning that a comparison between bacterial cellulose and the PLA electrospun nanofiber mats revealed a remarkable difference where the PLA nanofiber showed a higher sensitivity in terms of NH_3 detection due to its porosity and surface area. Furthermore, because of transparency, the colorimetric detection of bacterial cellulose is more difficult when compared with the PLA electrospun mats.

Additionally, the sensitivity and selectivity of the proposed sensor with various types of organic compounds were thoroughly explored. Likewise, the color change of the proposed sensor during the spoilage of the fresh meat sample was widely studied. Moreover, the proposed sensor was applied to fresh beef in two different conditions (room temperature and fridge condition) in order to study the efficiency of developed sensor for real situations in meat packaging. As observed, the colorimetric sensor monitored the freshness of beef extremely well and completely changed color before the threshold of beef spoilage.

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

6.1 Summary

In this research we investigated the development of a novel, colorimetric sensor for volatile detection and the possibility of its integration into real applications in meat packaging. Furthermore, this work emphasized the importance of presenting a novel colorimetric detector including the anthocyanin as a detector agent and PLA for the fabrication of an efficient sensor for use in the intelligent packaging industry.

In this chapter we conclude the outcomes achieved from this project, with respect to the results that is published in the journal article. The focus of this project was dedicated to examining a proof of concept for the detection of volatile compounds. In this case, the functionality of the PLA electrospun platform embedded with a novel pH colorimetric dye extracted from red cabbage was explored as a visual platform for detecting volatile compounds. The developed sensor was then exposed to different concentrations of NH_3.

By considering the production of TVB-N as a result of bacterial growth during the spoilage of meat, the proposed method was examined by using real meat and tested for the detection of meat spoilage by taking to the account the sensitive, selective and rapid detection parameters. The main outcomes are listed as follows:

Due to the interaction of the anthocyanin molecules immobilized onto the electrospun nanofibrous mats with NH_3 vapor in an acid-base reaction, the pH of anthocyanin changes and consequently the color of sensor varied due to the resin which is discussed in Chapter 2. The same phenomena occurred when the sensor was applied to the meat spoilage experiment.

The proposed sensor illustrated a high sensitivity though the determination of meat freshness based on the bacterial growth in beef and its color completely changed from pink to blueish green.

6.2 Recommendations and Future Work

The following topics would be of interest as future work:

- Due to the dependency of TVB-N concentration in the head space of the meat package on TVC, it is important to define a correlation between them as it would be beneficial in terms of more sensitive detection.
- Investigate the application of an applied sensor for monitoring the freshness of different protein-based products such as fish and poultry.
- Study the effect of different characterizations of PLA electrospun substrates on the sensitivity of the applied sensor.

REFERENCES

1. Burkepile, D.E., et al., *Chemically mediated competition between microbes and animals: microbes as consumers in food webs.* Ecology, 2006. **87**(11): p. 2821-2831.
2. Yam, K.L., P.T. Takhistov, and J. Miltz, *Intelligent packaging: concepts and applications.* Journal of food science, 2005. **70**(1): p. R1-R10.
3. Hernández-Cázares, A.S., M.-C. Aristoy, and F. Toldrá, *Hypoxanthine-based enzymatic sensor for determination of pork meat freshness.* Food chemistry, 2010. **123**(3): p. 949-954.
4. Mandenius, C., et al., *Monitoring and control of enzymic sucrose hydrolysis using on-line biosensors.* Applied microbiology and biotechnology, 1985. **21**(3-4): p. 135-142.
5. Manesh, K.M., et al., *Fabrication of functional nanofibrous ammonia sensor.* IEEE Transactions on Nanotechnology, 2007. **6**(5): p. 513-518.
6. Khattab, T.A., S. Abdelmoez, and T.M. Klapötke, *Electrospun nanofibers from a tricyanofuran-based molecular switch for colorimetric recognition of ammonia gas.* Chemistry–A European Journal, 2016. **22**(12): p. 4157-4163.
7. Kuswandi, B., et al., *A novel colorimetric food package label for fish spoilage based on polyaniline film.* Food Control, 2012. **25**(1): p. 184-189.
8. Gupta, B. and S. Sharma, *A long-range fiber optic pH sensor prepared by dye doped sol-gel immobilization technique.* Optics communications, 1998. **154**(5-6): p. 282-284.
9. Chang, Y.-C., et al., *Development of regenerative dye impregnated mesoporous silica materials for assessing exposure to ammonia.* Journal of the Air & Waste Management Association, 2012. **62**(7): p. 838-845.
10. Hoang, A.T., et al., *Sensitive naked-eye detection of gaseous ammonia based on dye-impregnated nanoporous polyacrylonitrile mats.* Sensors and Actuators B: Chemical, 2016. **230**: p. 250-259.
11. Golasz, L.B., J.d. Silva, and S.B.d. Silva, *Film with anthocyanins as an indicator of chilled pork deterioration.* Food Science and Technology, 2013. **33**: p. 155-162.
12. GIUSTI, M.M., et al., *Anthocyanin pigment composition of red radish cultivars as potential food colorants.* Journal of Food Science, 1998. **63**(2): p. 219-224.
13. Gao, Y., et al., *Polyaniline nanotubes prepared using fiber mats membrane as the template and their gas-response behavior.* The Journal of Physical Chemistry C, 2008. **112**(22): p. 8215-8222.

14. Ding, B., et al., *Electrospun nanomaterials for ultrasensitive sensors.* Materials Today, 2010. **13**(11): p. 16-27.
15. Xu, Q., et al., *Polydiacetylene-based colorimetric and fluorescent chemosensor for the detection of carbon dioxide.* Journal of the American Chemical Society, 2013. **135**(47): p. 17751-17754.
16. Kong, J., et al., *Nanotube molecular wires as chemical sensors.* science, 2000. **287**(5453): p. 622-625.
17. Yamada, Y., et al., *Nitrogen oxides sensing characteristics of Zn2SnO4 thin film.* Sensors and Actuators B: Chemical, 1998. **49**(3): p. 248-252.
18. Persaud, K. and G. Dodd, *Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose.* Nature, 1982. **299**(5881): p. 352.
19. Slater, J.M., et al., *Gas and vapour detection with poly (pyrrole) gas sensors.* Analyst, 1992. **117**(8): p. 1265-1270.
20. Slater, J.M., J. Paynter, and E. Watt, *Multi-layer conducting polymer gas sensor arrays for olfactory sensing.* Analyst, 1993. **118**(4): p. 379-384.
21. Schierbaum, K., U. Weimar, and W. Göpel, *Comparison of ceramic, thick-film and thin-film chemical sensors based upon SnO2.* Sensors and Actuators B: Chemical, 1992. **7**(1-3): p. 709-716.
22. Savage, N., et al., *Composite n–p semiconducting titanium oxides as gas sensors.* Sensors and Actuators B: Chemical, 2001. **79**(1): p. 17-27.
23. Varghese, O., et al., *Gas sensing characteristics of multi-wall carbon nanotubes.* Sensors and Actuators B: Chemical, 2001. **81**(1): p. 32-41.
24. Janata, J., *Principles of chemical sensors*. 2010: Springer Science & Business Media.
25. Ahmed, M.U., M.M. Hossain, and E. Tamiya, *Electrochemical biosensors for medical and food applications.* Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis, 2008. **20**(6): p. 616-626.
26. Xie, B., K. Ramanathan, and B. Danielsson, *Mini/micro thermal biosensors and other related devices for biochemical/clinical analysis and monitoring.* TrAC Trends in Analytical Chemistry, 2000. **19**(5): p. 340-349.
27. Rakow, N.A. and K.S. Suslick, *A colorimetric sensor array for odour visualization.* Nature, 2000. **406**(6797): p. 710.

28. Martinez, A.W., et al., *Patterned paper as a platform for inexpensive, low-volume, portable bioassays.* Angewandte Chemie International Edition, 2007. **46**(8): p. 1318-1320.
29. VS, A.P., et al., *Colorimetric sensors for rapid detection of various analytes.* Materials Science and Engineering: C, 2017. **78**: p. 1231-1245.
30. Pourjavaher, S., et al., *Development of a colorimetric pH indicator based on bacterial cellulose nanofibers and red cabbage (Brassica oleraceae) extract.* Carbohydrate polymers, 2017. **156**: p. 193-201.
31. Bethke, K., et al., *Functionalized cellulose for water purification, antimicrobial applications, and sensors.* Advanced Functional Materials, 2018. **28**(23): p. 1800409.
32. Zor, E., *Silver nanoparticles-embedded nanopaper as a colorimetric chiral sensing platform.* Talanta, 2018. **184**: p. 149-155.
33. Cheng, W., S. Dong, and E. Wang, *Iodine-induced gold-nanoparticle fusion/fragmentation/aggregation and iodine-linked nanostructured assemblies on a glass substrate.* Angewandte Chemie International Edition, 2003. **42**(4): p. 449-452.
34. Xia, Y., J. Si, and Z. Li, *Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review.* Biosensors and Bioelectronics, 2016. **77**: p. 774-789.
35. Schoolaert, E., R. Hoogenboom, and K. De Clerck, *Colorimetric nanofibers as optical sensors.* Advanced Functional Materials, 2017. **27**(38): p. 1702646.
36. Doong, R.-A. and H.-C. Tsai, *Immobilization and characterization of sol–gel-encapsulated acetylcholinesterase fiber-optic biosensor.* Analytica Chimica Acta, 2001. **434**(2): p. 239-246.
37. Gong, T., et al., *A facile fabrication of colorimetric graphene oxide reflecting films for ultrasensitive optical gas sensing.* Sensors and Actuators B: Chemical, 2018. **261**: p. 83-90.
38. Chen, X. and Z. Gu, *Absorption-type optical pH sensitive film based on immobilized purple cabbage pigment.* Sensors and Actuators B: Chemical, 2013. **178**: p. 207-211.
39. Gopalan, A., et al. *Fabrication of functional nanofibrous ammonia sensor*. in *2006 Sixth IEEE Conference on Nanotechnology*. 2006. IEEE.

40. Ji, S., Y. Li, and M. Yang, *Gas sensing properties of a composite composed of electrospun poly (methyl methacrylate) nanofibers and in situ polymerized polyaniline.* Sensors and Actuators B: Chemical, 2008. **133**(2): p. 644-649.
41. Pinto, N.J., et al., *Electric response of isolated electrospun polyaniline nanofibers to vapors of aliphatic alcohols.* Sensors and Actuators B: Chemical, 2008. **129**(2): p. 621-627.
42. Yang, M., et al., *Fabrication and photoelectric oxygen sensing characteristics of electrospun Co doped ZnO nanofibres.* Applied Physics A, 2007. **89**(2): p. 427-430.
43. Hoang, A.T., Y.B. Cho, and Y.S. Kim, *A strip array of colorimetric sensors for visualizing a concentration level of gaseous analytes with basicity.* Sensors and Actuators B: Chemical, 2017. **251**: p. 1089-1095.
44. Hu, M., et al., *A fluorescent and colorimetric sensor based on a porphyrin doped polystyrene nanoporous fiber membrane for HCl gas detection.* RSC Advances, 2017. **7**(43): p. 26849-26856.
45. Yurova, N.S., et al., *Functional electrospun nanofibers for multimodal sensitive detection of biogenic amines in food via a simple dipstick assay.* Analytical and bioanalytical chemistry, 2018. **410**(3): p. 1111-1121.
46. Schoolaert, E., et al., *Blend electrospinning of dye-functionalized chitosan and poly (ε-caprolactone): towards biocompatible pH-sensors.* Journal of Materials Chemistry B, 2016. **4**(26): p. 4507-4516.
47. Marega, C., et al., *Polyvinyl alcohol electrospun nanofibers containing Ag nanoparticles used as sensors for the detection of biogenic amines.* Nanotechnology, 2015. **26**(7): p. 075501.
48. Hu, M., et al., *Sensitive and fast optical HCl gas sensor using a nanoporous fiber membrane consisting of poly (lactic acid) doped with tetraphenylporphyrin.* Microchimica Acta, 2016. **183**(5): p. 1713-1720.
49. Crosland, M.P., *Gay-Lussac: scientist and bourgeois*. 2004: Cambridge University Press.
50. Wang, X., et al., *Electrospun nanofibrous membranes for highly sensitive optical sensors.* Nano letters, 2002. **2**(11): p. 1273-1275.
51. Dargaville, T. and B. Farrugia, *J. a. Broadbent, S. Pace, Z. Upton, NH Voelcker.* Biosens. Bioelectron, 2013. **41**: p. 30.

52. Fantini, D. and L. Costa, *Dye, fluorophores and pigment coloration of nanofibers produced by electrospinning.* Polymers for advanced technologies, 2009. **20**(2): p. 111-121.
53. Liang, X., et al., *Efficient method for fabrication of fluorescein derivative/PDAC composite nanofibers and characteristics of their photoluminescent properties.* European Polymer Journal, 2008. **44**(10): p. 3156-3162.
54. Frenot, A. and I.S. Chronakis, *Polymer nanofibers assembled by electrospinning.* Current opinion in colloid & interface science, 2003. **8**(1): p. 64-75.
55. Wang, X., et al., *Colorimetric sensor strips for formaldehyde assay utilizing fluoral-p decorated polyacrylonitrile nanofibrous membranes.* Analyst, 2013. **138**(17): p. 5129-5136.
56. Schmitt, K., et al. *Colorimetric sensor for bad odor detection using automated color correction*. in *Smart Sensors, Actuators, and MEMS VIII*. 2017. International Society for Optics and Photonics.
57. O'Toole, M., et al., *Inkjet printed LED based pH chemical sensor for gas sensing.* Analytica Chimica Acta, 2009. **652**(1-2): p. 308-314.
58. Byrne, L., K.T. Lau, and D. Diamond, *Monitoring of headspace total volatile basic nitrogen from selected fish species using reflectance spectroscopic measurements of pH sensitive films.* Analyst, 2002. **127**(10): p. 1338-1341.
59. Van der Schueren, L., et al., *The development of polyamide 6.6 nanofibres with a pH-sensitive function by electrospinning.* European Polymer Journal, 2010. **46**(12): p. 2229-2239.
60. Mudabuka, B., et al., *A colorimetric probe for ascorbic acid based on copper-gold nanoparticles in electrospun nylon.* Microchimica Acta, 2014. **181**(3-4): p. 395-401.
61. Van der Schueren, L., et al., *Polycaprolactone and polycaprolactone/chitosan nanofibres functionalised with the pH-sensitive dye Nitrazine Yellow.* Carbohydrate polymers, 2013. **91**(1): p. 284-293.
62. Poltue, T., et al., *Nickel (II) ions sensing properties of dimethylglyoxime/poly (caprolactone) electrospun fibers.* Materials Letters, 2011. **65**(14): p. 2231-2234.
63. Kim, S.-H. and J.-S. Bae, *Halochromic chemosensor prepared by pyran-based nanofibers.* Fibers and Polymers, 2013. **14**(12): p. 1981-1984.

64. Hu, L., et al., *Br-PADAP embedded in cellulose acetate electrospun nanofibers: Colorimetric sensor strips for visual uranyl recognition.* Journal of hazardous materials, 2017. **329**: p. 205-210.

65. Saithongdee, A., N. Praphairaksit, and A. Imyim, *Electrospun curcumin-loaded zein membrane for iron (III) ions sensing.* Sensors and Actuators B: Chemical, 2014. **202**: p. 935-940.

66. Prietto, L., et al., *Ultrafine fibers of zein and anthocyanins as natural pH indicator.* Journal of the Science of Food and Agriculture, 2018. **98**(7): p. 2735-2741.

67. Hu, Y., et al., *Effective strategy for colorimetric and fluorescence sensing of phosgene based on small organic dyes and nanofiber platforms.* ACS applied materials & interfaces, 2016. **8**(34): p. 22246-22252.

68. Ji, X., et al., *"Ready-to-use" hollow nanofiber membrane-based glucose testing strips.* Analyst, 2014. **139**(24): p. 6467-6473.

69. De Almeida, L., et al., *A novel colorimetric sensor strip for the detection of glyphosate in water.* Sensors and Actuators B: chemical, 2015. **206**: p. 357-363.

70. Geltmeyer, J., et al., *Dye modification of nanofibrous silicon oxide membranes for colorimetric HCl and NH3 sensing.* Advanced Functional Materials, 2016. **26**(33): p. 5987-5996.

71. Koppmann, R., *Volatile organic compounds in the atmosphere*. 2007: Wiley Online Library.

72. Joseph, P., et al., *Colorimetric sensors for rapid detection of various analytes.* Materials Science and Engineering: C, 2017. **78**: p. 1231-1245.

73. Kaneki, N., et al., *Measurement of pork freshness using potentiometric sensor.* Talanta, 2004. **62**(1): p. 215-219.

74. Zhang, C., D.P. Bailey, and K.S. Suslick, *Colorimetric sensor arrays for the analysis of beers: A feasibility study.* Journal of agricultural and food chemistry, 2006. **54**(14): p. 4925-4931.

75. Castro, P., et al., *Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice.* Food Control, 2006. **17**(4): p. 245-248.

76. Urmila, K., et al., *Quantifying of total volatile basic nitrogen (TVB-N) content in chicken using a colorimetric sensor array and nonlinear regression tool.* Analytical Methods, 2015. **7**(13): p. 5682-5688.
77. Silva-Pereira, M.C., et al., *Chitosan/corn starch blend films with extract from Brassica oleraceae (red cabbage) as a visual indicator of fish deterioration.* LWT-Food Science and Technology, 2015. **61**(1): p. 258-262.
78. Dong, S., et al., *Broad range pH sensor based on sol–gel entrapped indicators on fibre optic.* Sensors and Actuators B: Chemical, 2008. **129**(1): p. 94-98.
79. Zhang, X., S. Lu, and X. Chen, *A visual pH sensing film using natural dyes from Bauhinia blakeana Dunn.* Sensors and Actuators B: Chemical, 2014. **198**: p. 268-273.
80. Pávai, M., J. Mihály, and A. Paszternák, *pH and CO 2 sensing by curcumin-coloured cellophane test strip.* Food analytical methods, 2015. **8**(9): p. 2243-2249.
81. He, J. and M.M. Giusti, *Anthocyanins: natural colorants with health-promoting properties.* Annual review of food science and technology, 2010. **1**: p. 163-187.
82. Francis, F.J. and P.C. Markakis, *Food colorants: anthocyanins.* Critical Reviews in Food Science & Nutrition, 1989. **28**(4): p. 273-314.
83. Oliveira, J., et al., *Grape anthocyanin oligomerization: A putative mechanism for red color stabilization?* Phytochemistry, 2014. **105**: p. 178-185.
84. Ananga, A., et al., *Production of anthocyanins in grape cell cultures: a potential source of raw material for pharmaceutical, food, and cosmetic industries.* The Mediterranean genetic code-grapevine and olive, 2013: p. 247-287.
85. Clifford, M.N., *Anthocyanins–nature, occurrence and dietary burden.* Journal of the Science of Food and Agriculture, 2000. **80**(7): p. 1063-1072.
86. Merken, H.M. and G.R. Beecher, *Measurement of food flavonoids by high-performance liquid chromatography: a review.* Journal of Agricultural and Food chemistry, 2000. **48**(3): p. 577-599.
87. Patil, G., et al., *Extraction, dealcoholization and concentration of anthocyanin from red radish.* Chemical Engineering and Processing: Process Intensification, 2009. **48**(1): p. 364-369.

88. Kapasakalidis, P.G., R.A. Rastall, and M.H. Gordon, *Extraction of polyphenols from processed black currant (Ribes nigrum L.) residues.* Journal of agricultural and food chemistry, 2006. **54**(11): p. 4016-4021.

89. Metivier, R., F. Francis, and F. Clydesdale, *Solvent extraction of anthocyanins from wine pomace.* Journal of Food Science, 1980. **45**(4): p. 1099-1100.

90. Amr, A. and E. Al-Tamimi, *Stability of the crude extracts of Ranunculus asiaticus anthocyanins and their use as food colourants.* International journal of food science & technology, 2007. **42**(8): p. 985-991.

91. Awika, J.M., L.W. Rooney, and R.D. Waniska, *Anthocyanins from black sorghum and their antioxidant properties.* Food Chemistry, 2005. **90**(1-2): p. 293-301.

92. Jin, Z., et al., *Application of nano-crystalline porous tin oxide thin film for CO sensing.* Sensors and Actuators B: Chemical, 1998. **52**(1-2): p. 188-194.

93. Zhang, L., et al., *Hydrothermal synthesis of hierarchical nanoparticle-decorated ZnO microdisks and the structure-enhanced acetylene sensing properties at high temperatures.* Sensors and Actuators B: Chemical, 2011. **158**(1): p. 144-150.

94. Matsumiya, M., et al., *Nano-structured thin-film Pt catalyst for thermoelectric hydrogen gas sensor.* Sensors and Actuators B: Chemical, 2003. **93**(1-3): p. 309-315.

95. Reneker, D.H. and I. Chun, *Nanometre diameter fibres of polymer, produced by electrospinning.* Nanotechnology, 1996. **7**(3): p. 216.

96. Matthews, J.A., et al., *Electrospinning of collagen nanofibers.* Biomacromolecules, 2002. **3**(2): p. 232-238.

97. Reneker, D.H., et al., *Bending instability of electrically charged liquid jets of polymer solutions in electrospinning.* Journal of Applied physics, 2000. **87**(9): p. 4531-4547.

98. Vega-Lugo, A.-C. and L.-T. Lim, *Electrospinning of soy protein isolate nanofibers.* Journal of Biobased Materials and Bioenergy, 2008. **2**(3): p. 223-230.

99. Haider, A., S. Haider, and I.-K. Kang, *A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology.* Arabian Journal of Chemistry, 2018. **11**(8): p. 1165-1188.

100. Deitzel, J.M., et al., *The effect of processing variables on the morphology of electrospun nanofibers and textiles.* Polymer, 2001. **42**(1): p. 261-272.

101. Kwak, S., et al., *Micro/nano multilayered scaffolds of PLGA and collagen by alternately electrospinning for bone tissue engineering.* Nanoscale research letters, 2016. **11**(1): p. 323.
102. Megelski, S., et al., *Micro-and nanostructured surface morphology on electrospun polymer fibers.* Macromolecules, 2002. **35**(22): p. 8456-8466.
103. Jarusuwannapoom, T., et al., *Effect of solvents on electro-spinnability of polystyrene solutions and morphological appearance of resulting electrospun polystyrene fibers.* European Polymer Journal, 2005. **41**(3): p. 409-421.
104. Macossay, J., et al., *Effect of needle diameter on nanofiber diameter and thermal properties of electrospun poly (methyl methacrylate).* Polymers for Advanced Technologies, 2007. **18**(3): p. 180-183.
105. Sun, B., et al., *Advances in three-dimensional nanofibrous macrostructures via electrospinning.* Progress in Polymer Science, 2014. **39**(5): p. 862-890.
106. Fong, H., et al., *Generation of electrospun fibers of nylon 6 and nylon 6-montmorillonite nanocomposite.* Polymer, 2002. **43**(3): p. 775-780.
107. Bae, H.-S., et al., *Fabrication of highly porous PMMA electrospun fibers and their application in the removal of phenol and iodine.* Journal of Polymer Research, 2013. **20**(7): p. 158.
108. Pelipenko, J., et al., *The impact of relative humidity during electrospinning on the morphology and mechanical properties of nanofibers.* International journal of pharmaceutics, 2013. **456**(1): p. 125-134.
109. Park, J.-Y. and I.-H. Lee, *Relative humidity effect on the preparation of porous electrospun polystyrene fibers.* Journal of nanoscience and nanotechnology, 2010. **10**(5): p. 3473-3477.
110. Laurencin, C.T., et al., *Recent patents on electrospun biomedical nanostructures: an overview.* Recent patents on biomedical engineering, 2008. **1**(1): p. 68-78.
111. Chew, S., et al., *The role of electrospinning in the emerging field of nanomedicine.* Current pharmaceutical design, 2006. **12**(36): p. 4751-4770.
112. Huang, Z.-M., et al., *A review on polymer nanofibers by electrospinning and their applications in nanocomposites.* Composites science and technology, 2003. **63**(15): p. 2223-2253.
113. Li, D. and Y. Xia, *Electrospinning of nanofibers: reinventing the wheel?* Advanced materials, 2004. **16**(14): p. 1151-1170.

114. Stitzel, J.D., et al., *Arterial smooth muscle cell proliferation on a novel biomimicking, biodegradable vascular graft scaffold.* Journal of biomaterials applications, 2001. **16**(1): p. 22-33.

115. Woo, K.M., V.J. Chen, and P.X. Ma, *Nano-fibrous scaffolding architecture selectively enhances protein adsorption contributing to cell attachment.* Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 2003. **67**(2): p. 531-537.

116. Wang, X., et al., *Synthesis and electrospinning of a novel fluorescent polymer PMMA-PM for quenching-based optical sensing.* Journal of Macromolecular Science, Part A, 2002. **39**(10): p. 1241-1249.

117. Ding, B., M. Yamazaki, and S. Shiratori, *Electrospun fibrous polyacrylic acid membrane-based gas sensors.* Sensors and Actuators B: Chemical, 2005. **106**(1): p. 477-483.

118. Ahmed, E. and C. Carlstrom, *Food microbiology: A laboratory manual.* A John Wiley & sons, Inc. Publications, 2003.

119. Howgate, P., et al., *Multilingual guide to EC freshness grades for fishery products, December 1992.* 1992.

120. Alexander, C., N. Gregson, and Z. Gille, *Food waste.* The handbook of food research, 2013. **1**: p. 471-483.

121. Avella, M., et al., *Nanocomposite sensors for food packaging*, in *Nanotechnological Basis for Advanced Sensors*. 2011, Springer. p. 501-510.

122. Mukhopadhyay, S., et al. *A Novel Bio-sensor for Non-invasive Meat Inspection*. in *SENSORS, 2006 IEEE*. 2006. IEEE.

123. Abad, E., et al., *Flexible tag microlab development: gas sensors integration in RFID flexible tags for food logistic.* Sensors and Actuators B: Chemical, 2007. **127**(1): p. 2-7.

124. Borchert, N.B., J.P. Kerry, and D.B. Papkovsky, *A CO2 sensor based on Pt-porphyrin dye and FRET scheme for food packaging applications.* Sensors and Actuators B: Chemical, 2013. **176**: p. 157-165.

125. Olafsdottir, G., et al., *Multisensor for fish quality determination.* Trends in food science & technology, 2004. **15**(2): p. 86-93.

126. Chandrasekhar, J., M. Madhusudhan, and K. Raghavarao, *Extraction of anthocyanins from red cabbage and purification using adsorption.* Food and bioproducts processing, 2012. **90**(4): p. 615-623.

127. Wang, X., et al., *A facile and highly sensitive colorimetric sensor for the detection of formaldehyde based on electro-spinning/netting nano-fiber/nets.* Sensors and Actuators B: Chemical, 2012. **163**(1): p. 186-193.

128. Wang, D., et al., *Enhanced formaldehyde sensing properties of hollow SnO2 nanofibers by graphene oxide.* Sensors and Actuators B: Chemical, 2017. **250**: p. 533-542.

129. You, M.-H., et al., *Colorimetric humidity sensors based on electrospun polyamide/CoCl 2 nanofibrous membranes.* Nanoscale research letters, 2017. **12**(1): p. 360.

130. Kanmani, P. and J.-W. Rhim, *Antimicrobial and physical-mechanical properties of agar-based films incorporated with grapefruit seed extract.* Carbohydrate Polymers, 2014. **102**: p. 708-716.

131. Yoshida, C.M., et al., *Chitosan biobased and intelligent films: Monitoring pH variations.* LWT-Food Science and Technology, 2014. **55**(1): p. 83-89.

132. Pereira Jr, V.A., I.N.Q. de Arruda, and R. Stefani, *Active chitosan/PVA films with anthocyanins from Brassica oleraceae (Red Cabbage) as time–temperature indicators for application in intelligent food packaging.* Food Hydrocolloids, 2015. **43**: p. 180-188.

133. Rukchon, C., et al., *Development of a food spoilage indicator for monitoring freshness of skinless chicken breast.* Talanta, 2014. **130**: p. 547-554.

134. Maciel, V.B.V., C.M. Yoshida, and T.T. Franco, *Chitosan/pectin polyelectrolyte complex as a pH indicator.* Carbohydrate polymers, 2015. **132**: p. 537-545.

135. Yoon, J., S.K. Chae, and J.-M. Kim, *Colorimetric sensors for volatile organic compounds (VOCs) based on conjugated polymer-embedded electrospun fibers.* Journal of the American Chemical Society, 2007. **129**(11): p. 3038-3039.

136. Mercante, L.A., et al., *Electrospinning-based (bio) sensors for food and agricultural applications: A review.* TrAC Trends in Analytical Chemistry, 2017. **91**: p. 91-103.

137. Liu, J., et al., *Films based on κ-carrageenan incorporated with curcumin for freshness monitoring.* Food hydrocolloids, 2018. **83**: p. 134-142.

138. Ma, Q., L. Du, and L. Wang, *Tara gum/polyvinyl alcohol-based colorimetric NH3 indicator films incorporating curcumin for intelligent packaging.* Sensors and Actuators B: Chemical, 2017. **244**: p. 759-766.

139. Listyarini, A., W. Sholihah, and C. Imawan. *A paper-based Colorimetric Indicator Label using Natural Dye for Monitoring Shrimp Spoilage*. in *IOP Conference Series: Materials Science and Engineering*. 2018. IOP Publishing.

140. Halász, K. and L. Csóka, *Black chokeberry (Aronia melanocarpa) pomace extract immobilized in chitosan for colorimetric pH indicator film application.* Food packaging and shelf life, 2018. **16**: p. 185-193.

141. Nguyen, L.T., et al., *Biological, chemical, and electronic applications of nanofibers.* Macromolecular materials and engineering, 2013. **298**(8): p. 822-867.

142. Veiga-Santos, P., C. Ditchfield, and C.C. Tadini, *Development and evaluation of a novel pH indicator biodegradable film based on cassava starch.* Journal of Applied Polymer Science, 2011. **120**(2): p. 1069-1079.

143. Martins, J.T., M.A. Cerqueira, and A.A. Vicente, *Influence of α-tocopherol on physicochemical properties of chitosan-based films.* Food hydrocolloids, 2012. **27**(1): p. 220-227.

144. Fuleki, T. and F. Francis, *Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in cranberries.* Journal of food science, 1968. **33**(1): p. 72-77.

145. Fuleki, T. and F. Francis, *Quantitative methods for anthocyanins. 2. Determination of total anthocyanin and degradation index for cranberry juice.* Journal of food science, 1968. **33**(1): p. 78-83.

146. Savoji, H., et al., *Plasma-etching for controlled modification of structural and mechanical properties of electrospun PET scaffolds.* Plasma Processes and Polymers, 2015. **12**(4): p. 314-327.

147. Yousefi, H., et al., *Cellulose nanofiber board.* Carbohydrate polymers, 2018. **187**: p. 133-139.

148. Chang, L.-Y., et al., *One-minute fish freshness evaluation by testing the volatile amine gas with an ultrasensitive porous-electrode-capped organic gas sensor system.* ACS sensors, 2017. **2**(4): p. 531-539.

149. Pacquit, A., et al., *Development of a smart packaging for the monitoring of fish spoilage.* Food Chemistry, 2007. **102**(2): p. 466-470.

150. RESILIENCE, B., *THE STATE OF FOOD SECURITY AND NUTRITION IN THE WORLD.* 2017.

151. Prietto, L., et al., *pH-sensitive films containing anthocyanins extracted from black bean seed coat and red cabbage.* LWT, 2017. **80**: p. 492-500.

152. Ding, B., et al., *Electrospun nanofibrous membranes coated quartz crystal microbalance as gas sensor for NH3 detection.* Sensors and Actuators B: Chemical, 2004. **101**(3): p. 373-380.

153. Bin Ding, M.K., *Electrospun nanofibrous polyelectrolytes membranes as high sensitive coatings for QCM-based gas sensors.* Nanotechnology at the Leading Edge, 2006: p. 1.