Talanta 134 (2015) 718–723

Contents lists available at ScienceDirect

Talanta

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

# Non-destructive detection of fish spoilage using a wireless basic volatile sensor

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ARTICLE INFO

*Article history:*
Received 2 October 2014
Received in revised form
16 December 2014
Accepted 17 December 2014
Available online 25 December 2014

*Keywords:*
Ammonia vapor
Fish spoilage
Food freshness
Inductive coupling
LC resonator
Passive RFID
pH-electrode
TVB-N
Volatiles
Wireless sensor

ABSTRACT

A hydrogel-pH-electrode based near-field passive volatile sensor is described for real-time monitoring of fish spoilage. The sensor employs a varactor-based LC resonator that can be interrogated remotely using inductive coupling. The sensor's resonant frequency varies in response to the basic volatile spoilage compounds (total volatile basic nitrogen, TVB-N) in the headspace of packaged fish. The sensor is shown to have a linear response to logarithm of the ammonia gas concentration with a detection limit of 0.001 mg $L^{-1}$ (1.5 ppm). Trials on tilapia at 24 °C and 4 °C, employing direct comparison of sensor measurements with microbial analysis, indicate that the sensor response is correlated with the bacterial growth pattern in fish samples. It is shown that the sensor can distinctly identify when the product rejection level ($10^7$ cfu $g^{-1}$ bacterial population) occurs for both 24 °C and 4 °C storage conditions. This demonstrates a potential for real-time monitoring of fish spoilage. The wireless sensor is suited to embedding in packaging material and does not require an integrated circuit, making it amenable to inexpensive mass production using printed electronic technology.



## 1. Introduction

Food spoilage is an important topic to both the consumer and food processing industry. It is not only a human health concern but also a major economic issue due to food wastage [1,2]. As the present global economy has lead to increased distance between the consumer and production zone, and subsequently a complex supply chain, new methods for monitoring food quality is needed [3,4]. Fish is a widely consumed food worldwide and there is a great interest among the food industries, retailers, consumers and their stakeholders to develop methods for evaluating fish freshness in real time [5]. Various approaches have been used to determine fish freshness. In the fish industry, specialized trained assessors evaluate freshness attributes, such as, appearance, color, smell and texture. A certain grading scheme is then used by compiling these qualities to produce a quality index [6]. This procedure is labor intensive and unreliable. As microbe growth is the main cause of fish quality degradation, total viable count (TVC) is considered as a definitive index for fish spoilage monitoring. After death, the number of microorganisms on the skin and gill surfaces increases gradually and spreads within various tissues. These microorganisms are known as spoilage organisms and are usually *Pseudomonas* spp. [5–8]. As the fish deteriorates due to the microorganisms, volatile compounds such as $(CH_3)_3N$ (trimethylamine or TMA), $(CH_3)_2NH$ (dimethylamine or DMA) and $NH_3$ (ammonia) are produced. These products are collectively known as TVB-N. Therefore, TVB-N levels are an indicator of fish freshness. Headspace methods are among the most reliable methods for volatile compound analysis. They consist of collection and concentration of the volatiles for subsequent chromatographic separation to identify and qualify the separated compounds [5,6,9–11]. Olafsdottir et al. provided a review of these methods in [6]. Unfortunately, these methods are time-consuming, and require trained personnel and laboratory equipment. They generally involve invasively breaching the package and therefore rendering the individual product useless. Further, this selective sampling does not offer any guarantee that the rest of the batch is fresh [6,11].

An array of metal oxide semiconductor (MOS) sensors have been used for detecting volatile compounds produced during spoilage of silver cup, salmon, haddock, cod, red fish, and so on [12–14]. MOS sensors are cheap, have high longevity and electronic simplicity. However, they require high temperature (200–500 °C) and substantial power to operate, and have limited selectivity [2,13]. Non-destructive tests such as fluorescence spectroscopy and nuclear imaging have also been used for determining fish freshness. The fish muscle exhibits intrinsic fluorescence and the intensity of this fluorescence decreases with storage time on ice [4]. Spectroscopic methods have so far not

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http://dx.doi.org/10.1016/j.talanta.2014.12.017
0039-9140/

proven sufficient to fully characterize the properties of fresh fish [6]. Nuclear imaging, a mature diagnosis method for analyzing organ and texture, has been applied to inspect dynamic changes of carp muscle to evaluate the fish freshness [15]. However, nuclear imaging is a costly technique [6]. As the pH of the fish changes during the spoilage process, a custom radio-frequency-identification (RFID) based pH sensor has been demonstrated for fish spoilage monitoring [2]. The sensor employs a pH-sensitive electrode pair which needs to be in contact with the fish, thus posing a risk of electrode fouling and food contamination. Monitoring ambient temperature during transportation and storage is an indirect method of promoting fish quality. However, this approach does not directly monitor the fish product and quality cannot be ensured. pH-sensitive dye and polyaniline (PANI)-based colorimetric sensors have been demonstrated as a method to monitor the spoilage of Cod, Cardinal, Roundnose grenadier and Milk fish [6,11]. These sensors change color in response to the TVB-N level in the headspace of the packaged fish. They are non-destructive, low cost and do not require contact with the fish sample, however, they require visual inspection and are not easily translated to qualitative values. Therefore, there is a pressing demand to develop an electronic based real-time fish spoilage monitoring device that is non-destructive, accurate, simple, low-cost, reliable and does not require contact with the fish.

In this article, we present the use of a hydrogel coated pH-electrode based near-field passive sensor for determining fish spoilage. In previous work, we reported a near field passive sensor for detecting basic volatile concentration [16]. It employs a varactor based passive LC resonator whose resonant frequency depends on the basic volatile concentration in its surrounding environment. During spoilage total volatile basic nitrogen (TVB-N) are produced gradually in the fish package and change in sensor's resonant frequency occurs. The sensor's resonant frequency is detected by measuring the impedance of an external interrogator coil that is inductively coupled to the sensor. We present experimental results of prototype sensors monitoring the spoilage of tilapia fish at 24 °C and 4 °C. Direct comparison is made with microbial analysis. The design of the sensor is simple and suited for inexpensive mass production using printed electronic technology [17]. As this sensor is wireless, passive and does not require any contact with the fish sample, it can be a low cost, non-destructive, consumer friendly and reliable alternative for fish spoilage monitoring in individual packages.

## 2. Experiment

### 2.1. Sensor fabrication

#### 2.1.1. Hydrogel-pH-electrode pair

The electrodes and hydrogel coating were prepared using the method described in [16]. Mixed metal oxide (MMO) and silver/silver chloride (Ag/AgCl) were chosen as the pH-sensitive electrode and the pH-insensitive reference electrode, respectively. A MMO electrode was chosen because of its commercial availability, low cost, and pH sensitivity [18]. The Ag/AgCl electrode was coated with immobilized electrolyte solution and protective Nafion layer. Ag/AgCl was selected as the pH-insensitive reference electrode because of its wide use as a reference electrode in industrial applications, simple construction and inexpensive manufacturing cost. A thin layer (2.5 mm thick) of hydrogel coating is placed on top of the electrodes and acts to contain the electrolyte. The amorphous hydrogel (Intrasite gel from Smith and Nephew) is a clear gel containing a modified carboxymethyl cellulose polymer, propylene glycol and water. It has an initial pH of ~7.1.

#### 2.1.2. Prototype sensor and interrogator

The sensor, as shown in Fig. 1a, was constructed using the MMO and Ag/AgCl electrodes. It was designed to have a resonant

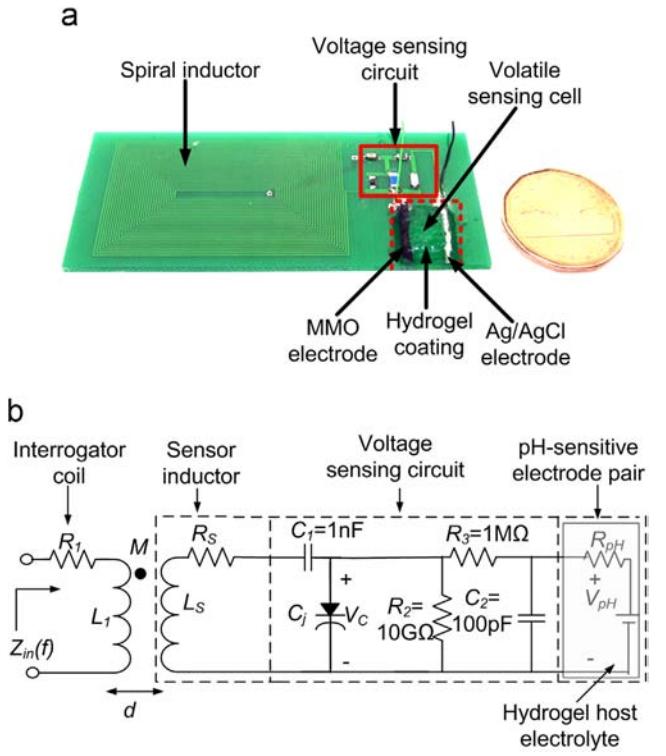


**Fig. 1.** (a) Prototype volatile absorption sensor with hydrogel coated MMO and Ag/AgCl electrodes. The prototype is fairly large and can be further miniaturized. (b) Equivalent circuit diagram of the passive sensor including the near-field coupled interrogator coil (interrogator not shown in Fig. 1a).

frequency, $f_0$, near 6 MHz. It was fabricated on a 8 cm × 3.5 cm FR4 printed circuit board (PCB) with a 27 turn rectangular coil inductor, surface-mount capacitors and resistors [19]. The inductor trace width, spacing and thickness are 0.254 mm, 0.254 mm and 0.15 mm, respectively, producing $L_S$=20.33 μH and $R_S$=9 Ω at 6 MHz. The junction capacitance of the varactor (NXP BB202) in the voltage sensing circuit, $C_j$, varied in the range of 35.04 pF - 22.95 pF for reverse bias voltages between 0 and 1 V, respectively. The hydrogel coated Ag/AgCl and MMO electrodes were connected to the positive and negative terminals of the voltage sensing circuit, respectively. The series resistance, $R_{pH}$ of the MMO and Ag/AgCl reference electrode pair when coated with the amorphous hydrogel was found to be approximately 500 kΩ. The interrogator coil was constructed of 5 turns of insulated copper wire with a self-resonant frequency, $f_{res}$=28.32 MHz.

An equivalent circuit diagram of the sensor is shown in Fig. 1b. In the remote sensor, a spiral inductor is connected in parallel with a voltage-dependent capacitor (varactor) based voltage sensing circuit and a hydrogel coated pH-sensitive electrode pair. The pH-sensitive electrode pair consists of a pH-sensitive electrode and a pH-insensitive reference electrode. The potential difference between the electrodes acts as a bias voltage across the varactor. $L_S$ is the inductance of the spiral inductor, $C(V_{pH})$ is the capacitance of the voltage sensing circuit and $V_{pH}$ is the potential difference developed across the electrodes. Basic volatiles produced in a closed environment are absorbed by the hydrogel. As a result, the hydrogel pH changes which in turn changes the voltage, $V_{pH}$, across the pH-sensitive electrode pair. The capacitance, $C(V_{pH})$ changes in response to the low frequency change of the biasing voltage, $V_{pH}$. The spiral inductor and capacitor form a resonant circuit with a resonant frequency, $f_0$, given by

$$f_0 = \frac{1}{2\pi\sqrt{L_S C(V_{pH})}}. \quad (1)$$

In this manner the sensor's resonant frequency is directly related to the basic volatile absorbed by the hydrogel. The resonant frequency was determined wirelessly by measuring the real part of the impedance of the interrogator coil when inductively coupled to the sensor inductor. The interrogator coil impedance was measured using an impedance analyzer (Agilent 4292A). Details of the sensor circuit and measurement of resonant frequency were explained in [16].

### 2.2. Sensor response to ammonia

The absorption of ammonia into the hydrogel and subsequent response of the sensor were evaluated by placing the sensor in a closed environment with different concentrations of $NH_3$. The test for each concentration was divided into two phases. In phase (i) the sensor was placed in a closed environment for 20 min with no volatile present. In phase (ii) a particular concentration of $NH_3$ was introduced in the environment. The two phase test method was discussed in detail in [16]. The mean laboratory temperature was 24.3 °C ($\sigma$=0.7 °C) throughout all experiments. In both phases the relative humidity was 100%. Since the sensor is not fully reversible [19], the electrodes were cleaned with DI water and coated with fresh hydrogel before beginning phase (i) of each test. The sensor inductor was aligned concentrically with the interrogator coil at a distance of 10 cm.

### 2.3. Fish spoilage trials

#### 2.3.1. Wireless tilapia spoilage test at 24 °C

Two fresh tilapia fish fillets were supplied by local fish retailer (Gimli fish supplier, Winnipeg) and transported to the lab in a sealed ice container. Aseptic techniques such as disposable gloves, autoclaved cutting board, autoclaved and flame sterilized scalpel, forceps were used to avoid sample contamination. One tissue sample of 25 g was removed from the fillets and placed in a 500 ml sterilized polycarbonate jar. A prototype wireless sensor (prepared according to Section 2.1) with fresh hydrogel coating was placed inside the jar. The jar was sealed with a polypropylene screw lid. The jar was kept at room temperature (24 °C) throughout the experiment. The sensor's resonant frequency was monitored wirelessly every 3 h for 33 h with the interrogator coil and impedance analyzer. The sensor inductor was aligned concentrically with the interrogator coil at a distance of 10 cm. Fig. 2 shows the experimental setup.

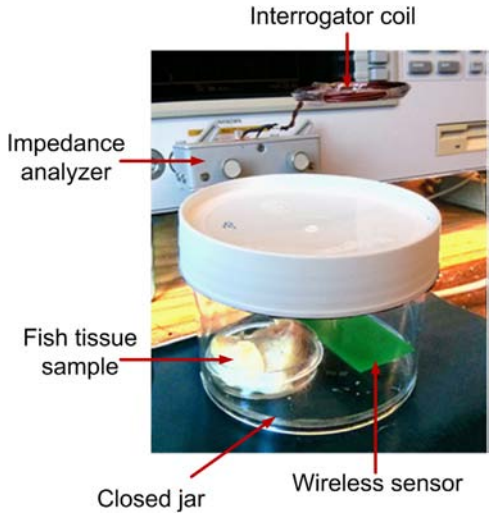


**Fig. 2.** Experimental setup for wireless tilapia spoilage test at 24 °C. A 25 g tissue sample of fish and the wireless sensor are placed in a 500 ml polycarbonate jar. The sensor's resonant frequency is measured using the interrogator coil connected to an impedance analyzer.

#### 2.3.2. Microbial analysis of tilapia samples at 24 °C

Simultaneously to the wireless spoilage trial, 12 samples of 25 g of fish tissue were removed from the same tilapia fillets, under the same aseptic conditions and placed in 12 polycarbonate jars. A fresh hydrogel coated pH-sensitive electrode pair was placed in each of these jars. The jars were sealed with polypropylene screw lids. Fig. 3 shows some of these jars. In this test direct measurement of the electrode potential, $V_{pH}$, was performed using wired connections to the hydrogel coated electrode pairs though the lids of the jars. As in the wireless test these samples were allowed to spoil at room temperature (24 °C). Every 3 h one jar was selected at random and the fish sample was taken out for microbial analysis with the first one done at zero hour to determine the initial microbial loads. Before the jar was opened for microbial analysis, voltages across all the hydrogel coated pH-sensitive electrode pairs were monitored. The electrode pair in the opened jar was discarded. For microbial analysis, total viable count (TVC) and *Pseudomonas* count were determined using a spread plate method on plate count agar (Oxoid CM0463) and agar base (Oxoid CM0559) with CFC selective supplement (Oxoid SR0103), respectively. Plates were counted after 48 h incubation at 30 °C. Bacterial population was correlated with wireless sensor measurements and voltages, $V_{pH}$, across the hydrogel coated electrode-pairs.

#### 2.3.3. Wireless tilapia spoilage test at 4 °C

The experimental procedure used for the fish spoilage test at 4 °C was similar to the one at 24 °C (Section 2.3.1.). Two tissue samples of 25 g were removed from the fillets and placed in two 500 ml sterilized polycarbonate jars, each with a prototype wireless sensor (prepared according to Section 2.1) with fresh hydrogel coating. The jars were sealed with Saran™ food wrap (PVDC with PP coating). The jars were kept in a refrigerator at 4 °C. The resonant frequencies of both sensors were monitored wirelessly every 8 h for 104 h with the interrogator coil and impedance analyzer. The sensor inductor was aligned concentrically with the interrogator coil at a distance of 10 cm. The test at 4 °C was done with Saran™ food wrap to imitate a realistic storage condition of fresh fish.

**Fig. 3.** Samples of tilapia tissue kept at 24 °C for microbial analysis. Each jar contains a 25 g tissue sample of fish and a hydrogel coated pH-sensitive electrode pair with wired connection to a voltmeter.

*2.3.4. Microbial analysis of tilapia samples at 4 °C*

Simultaneously to the above spoilage trial at 4 °C, 14 samples of 25 g of fish tissue were removed from the same tilapia fillets, under the same aseptic conditions and placed in 14 polycarbonate jars. A fresh hydrogel coated pH-sensitive electrode pair was placed in each of these. Direct wired connections to the hydrogel coated electrode pairs were made to monitor $V_{pH}$. The jars were sealed with Saran™ food wrap (PVDC with PP coating). These samples were also allowed to spoil at 4 °C inside the refrigerator. At 8 h intervals, and at the same time the wireless sensor response was recorded, one of the samples was removed for microbial analysis. The first one was done at zero hour to determine the initial microbial loads. Before the jar was opened for microbial analysis, voltages across all hydrogel coated pH-sensitive electrode pairs were monitored. The electrode sample in the opened jar was discarded. The microbial analysis was done the same manner as described in Section 2.3.2.

## 3. Results

### 3.1. Sensor response to ammonia

As described in Section 2.2., the resonant frequency of the sensor was measured as a function of time for different concentrations of $NH_3$. The result is shown in Fig. 4. It can be seen that when a fixed concentration of $NH_3$ was introduced to the sensor environment, the resonant frequency decreased, eventually stabilizing at an equilibrium value related to that concentration. It took approximately 20 min for the sensor to reach the equilibrium value after being introduced to the $NH_3$ irrespective of concentration. This response time is adequate for fish spoilage monitoring as the increase in total volatile basic nitrogen (TVB-N) in the fish package will occur over a period of several hours to days. The response time can be reduced by decreasing the thickness of the hydrogel coating [16].

To examine repeatability, for each concentration of $NH_3$, the response test was performed three times and results are shown in Fig. 5. Each data point consists of an average of 10 resonant frequency measurements, taken at 2 min intervals after the sensor reaches its equilibrium state (20 min after exposure). The resonant frequency exhibits a linear relationship with the logarithm of $NH_3$ concentration. Fig. 5 shows that $NH_3$ concentration can be measured with an accuracy of 13% (based on maximum deviation from the linear fit). Using the standard deviation of the resonant frequency during the initial zero volatile period (first 20 min of Fig. 4) the sensor has a frequency noise floor of 7.4 kHz. This corresponds to a detection limit of 0.001 mg $L^{-1}$ (1.5 ppm).

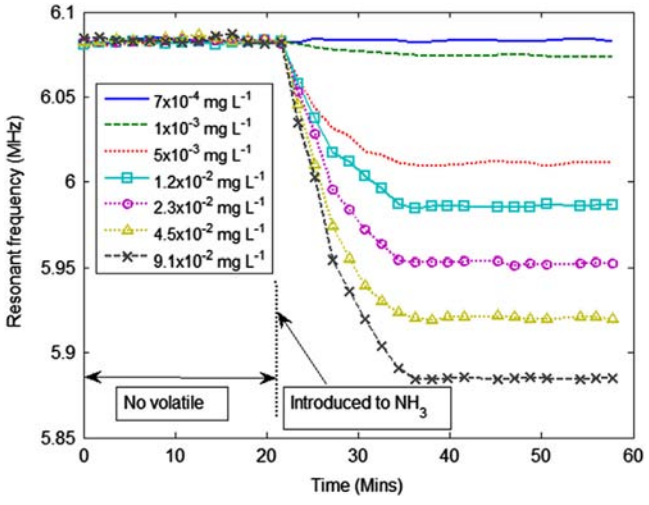


**Fig. 4.** Resonant frequency of the sensor measured for different concentrations of $NH_3$ at 24 °C.

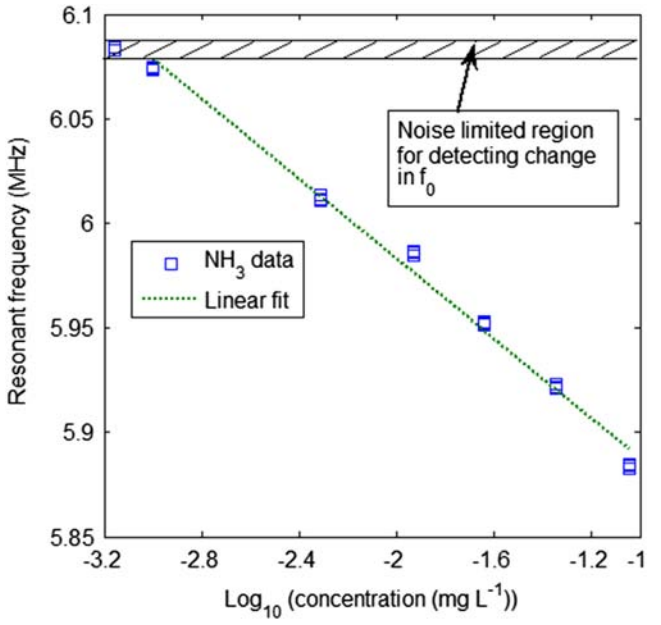


**Fig. 5.** Resonant frequency of the sensor after reaching equilibrium state as a function of $NH_3$ concentration at 24 °C (20 mins. after initial exposure refer to Fig. 4). For each concentration 3 measurements are shown, each consisting of an average of 10 measurements at 2 mins. intervals.

Previous studies showed that the slope of response curve and the $V_{pH}$ value for zero acidic/basic volatiles concentration decreased with decrease of temperature [16]. This is due to temperature sensitivity of the pH-sensitive electrode pair and the effect of temperature on the mobility of ions inside the hydrogel [20]. One mechanism is the temperature dependence of the gas diffusion coefficient which increases the sensor's response time with decrease of temperature [21]. Also, under low relative humidity conditions the hydrogel dehydrates and does not act like an electrolyte for the pH-sensitive electrode pair [22]. Previous studies also showed that the sensor requires a relative humidity between 100% to 45% to perform properly [16]. Generally in a sealed fish package environment the relative humidity is higher than this level [23].

### 3.2. Fish spoilage trial at 24 °C

Fig. 6 shows the sensor's resonant frequency and the TVC and *Pseudomonas* count for fish spoilage trials at 24 °C. The sensor response is correlated with the bacterial population. The resonant frequency of the wireless sensor (in the same container as the tilapia tissue sample) decreased with increasing total volatile basic nitrogen (TVB-N) generated during tilapia spoilage. The sensor was monitored every 3 h until no further substantial change in resonant frequency was observed. The TVC slowly increased from a level near $10^5$ cfu $g^{-1}$ during the initial 9 h and then rose sharply before stabilizing at approximately $10^9$ cfu $g^{-1}$ at 27 h. Initially the *Pseudomonas* count was approximately 75% of the TVC count and rose to near 100% at after 12 h. Similarly to the TVC count, the *Pseudomonas* count increased sharply and stabilized at approximately $10^9$ cfu $g^{-1}$ at 27 h. Koutsoumanis and Olafsdottir *et al.* both reported TVC and *Pseudomonas* values of $10^7$ cfu $g^{-1}$ as the level where fresh fish samples have reached their end of shelf life [6,24]. This level occurred at 15 h in our experiment. This end-of-shelf-life level can be clearly identified by the 80 kHz change in sensor resonant frequency.

Results of the microbial analysis and wired sensor response at 24 °C are shown in Fig. 7. Here the TVC and *Pseudomonas* count,

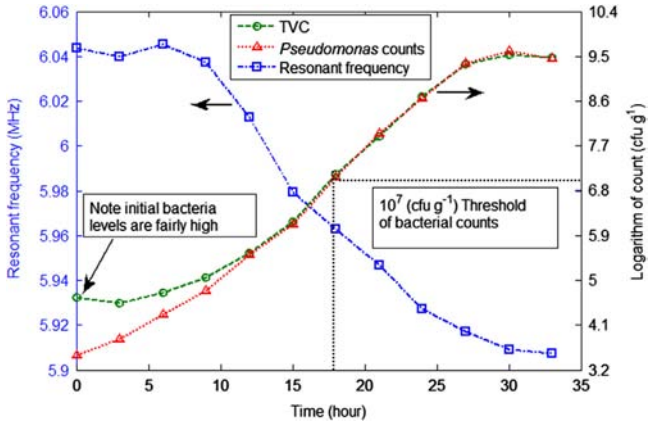


**Fig. 6.** Resonant frequency of the sensor and bacterial population in fresh tilapia kept at 24 °C over a period of 1.5 days. Bacterial data are the average of two replicates.

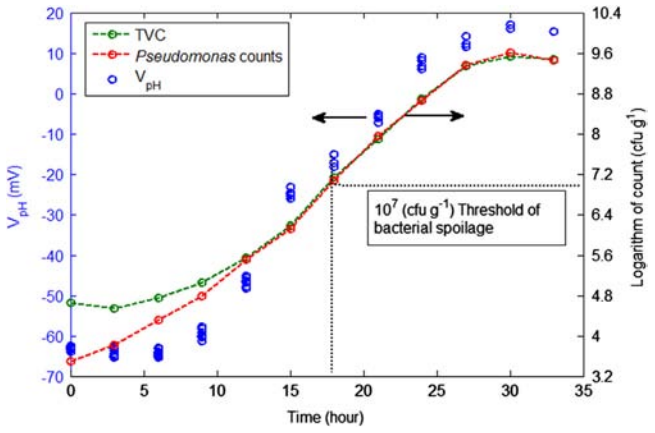


**Fig. 7.** Measured voltages across all 12 hydrogel coated pH-sensitive electrode pairs and change in bacterial population for tilapia at 24 °C. Bacterial data are an average of two replicates.

and the voltage, $V_{pH}$, measured across the hydrogel coated pH-sensitive electrode pairs are provided. In this study, the sensors were in the same container as the 12 tilapia tissue samples used for microbial analysis and were measured every 3 h. Thus at the beginning of the trial 12 $V_{pH}$ data points are indicated. The data points decreased every 3 h as samples were opened and used for microbial analysis. The response, $V_{pH}$ values, from all 12 wired sensors are very similar with a deviation of less than $\pm$2.8 mV and are correlated with the change in the bacterial population of the fish samples. The similarity of all 12 wired sensor responses demonstrates that the hydrogel-pH-sensitive electrode sensor can be fabricated reproducibly. An initial trial (not shown here), performed in the same manner with wired sensors at 24 °C, showed an analogous relationship between sensor response and bacterial population over time.

### 3.3. Fish spoilage trial at 4 °C

Fig. 8 shows the resonant frequency and the TVC and *Pseudomonas* count for fish spoilage trials at 4 °C. Two jars, each with a tilapia sample and a wireless sensor were used. The responses of both sensors are correlated with the change in bacterial population. The resonant frequencies of the wireless sensors decreased with increasing total volatile basic nitrogen (TVB-N) generated during tilapia spoilage. The sensors were monitored every 8 h until no further substantial change in resonant frequencies was observed. The TVC slowly increased from a level near $10^4$ cfu g$^{-1}$

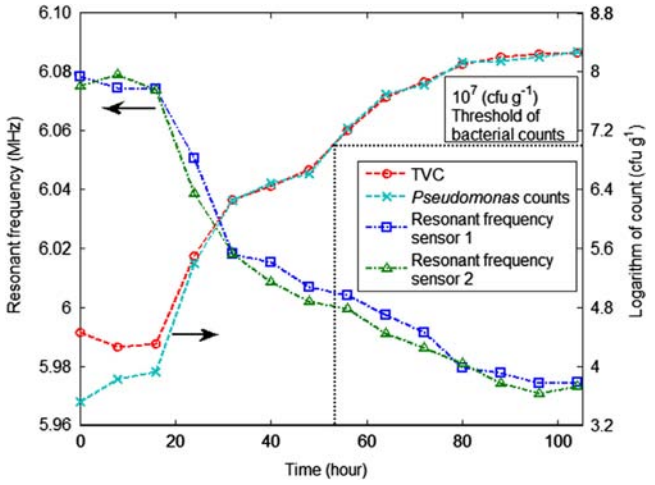


**Fig. 8.** Resonant frequencies of the two sensors and bacterial population in fresh tilapia kept at 4 °C over a period of 4.5 days. Bacterial data are the average of two replicates.

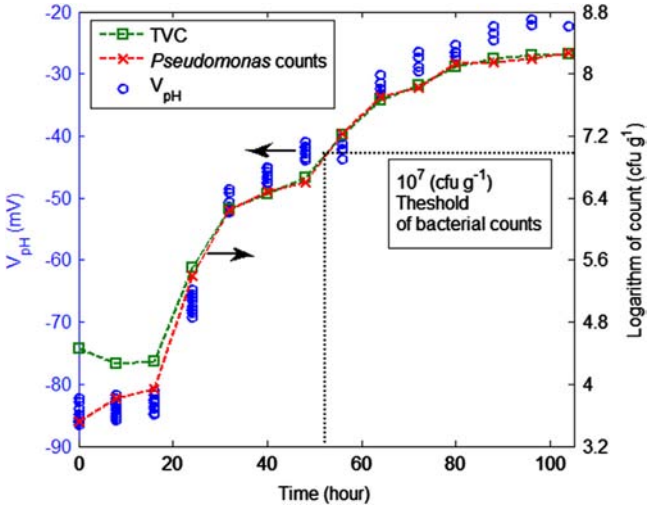


**Fig. 9.** Measured voltages across all 14 hydrogel coated pH-sensitive electrode pairs and change in bacterial population for tilapia at 4 °C. Bacterial data are the average of two replicates.

during the initial 16 h and then rose sharply before stabilizing at approximately $10^8$ cfu g$^{-1}$ at 88 h. Initially the *Pseudomonas* count was approximately 78% of the TVC count and rose to near 100% after 32 h. Similarly to the TVC count, the *Pseudomonas* count increased sharply and stabilized at approximately $10^8$ cfu g$^{-1}$ at 88 h. The end-of-shelf-life level ($10^7$ cfu g$^{-1}$) occurred at 48 h in our experiment. This level can be clearly identified by the 72 kHz change in sensor resonant frequency. The two individual sample tests performed with the wireless sensors showed a very similar resonant frequency behavior, with a deviation of less than $\pm$4.16 kHz, and are correlated with the change in the bacterial population of the fish samples. Similarity of the two wireless sensor responses demonstrates the reproducibility of the sensor.

Results of the microbial analysis and wired sensor response at 4 °C are shown in Fig. 9. The TVC and *Pseudomonas* count, and the voltage, $V_{pH}$, measured across the hydrogel coated pH-sensitive electrode pairs are provided. The sensors were in the same container as the tilapia tissue samples used for microbial analysis and were measured every 8 h. The response, $V_{pH}$ values, from all 14 wired sensors are very similar with a deviation of less than $\pm$3 mV and are correlated with the change in the bacterial population of the fish samples.

## 4. Discussion and conclusion

We demonstrated a hydrogel-pH-electrode based wireless sensor as a possible method for food freshness monitoring of packaged fish. The sensor employs a near-field coupled passive LC resonator whose resonant frequency is sensitive to basic volatile concentration. It exhibits a linear response that varies with the logarithm of ammonia concentration and has a detection limit of 0.001 mg $L^{-1}$ (1.5 ppm). Trials of monitoring the spoilage of tilapia over several days at 24 °C and 4 °C showed that the sensor response was correlated with the bacterial growth in the fish tissue. The sensor was able to identify when the TVC exceeded a level of $10^7$ cfu $g^{-1}$, indicative of end-of-shelf-life.

The sensor demonstrated repeatable results for a constant temperature. However, the sensor's response baseline shifted when temperature decreased from 24 °C to 4 °C, along with a slight change in sensitivity. This temperature dependence is typical of pH-electrode pair sensors. Previous studies of this particular sensor showed the $V_{pH}$ baseline decreased by 18 mV and the sensitivity decreased by 5 mV/log (concentration (ppm)) when temperature decreased from 24 °C to 4 °C [16]. This behavior matches the results in Figs. 7 and 9. Thus, in order to reliably correlate bacteria count with sensor response, the temperature of the sample must be known or a temperature compensation method should be employed [20]. In addition the sensor required a relative humidity greater than 45% in order to maintain the electrolyte in the hydrogel [16]. The relative humidity constraint can be alleviated by using an encapsulation membrane, impermeable to water vapor, or an alternate host electrolyte.

The sensor does not require a custom integrated circuitry, making it amenable to low cost mass production using printed electronics technology. The passive components: inductor coil, capacitors and resistors are amenable to several flexible substrate printing approaches [25,26]. The Ag/AgCl/nafion reference electrode can be fabricated using the inkjet printing/screen printing method [17], as can the pH-sensitive MMO electrode [27]. Even though printed transistor and diode technologies are available, printed RF varactors are still a research topic [26]. The electrodes proposed for this sensor are not toxic and have been previously used in food and biological media applications [2,28]. The hydrogel used for coating the electrodes is regularly used for medical usage and is biocompatible, even though an encapsulation method still needs to be deployed [29]. Additionally polyamide substrates have been previously used as non-toxic material and would be compatible with food packaging materials by attachment to the inside of the fish package wrap.

## Acknowledgments

The authors would like to thank Xiang Chen, Xuan Zhang and Maria Beijaflor from the Department of Food Science, University of Manitoba for their help during the microbial analysis of fish samples. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

## References

[1] R.L. Scharff, J. Food Prot. 75 (2012) 123–131.
[2] W.D. Huang, S. Deb, Y.S. Seo, S. Rao, M. Chiao, J.C. Chiao, IEEE Sens. J 12 (2012) 487–495.
[3] K.G. Ong, J.S. Bitler, C.A. Grimes, L.G. Puckett, L.G. Bachas, Sensors 2 (2002) 219–232.
[4] É. Dufour, J.P. Frencia, E. Kane, Food Res. Int. 36 (2003) 415–423.
[5] A. Pacquit, K.T. Laua, H. McLaughlin, J. Frisby, B. Quilty, D. Diamond, Talanta 69 (2005) 515–520.
[6] G. Olafsdóttir, E. Martinsdóttir, J. Oehlenschläger, P. Dalgaard, B. Jensen, I. Undeland, I.M. Mackie, G. Henehan, J. Nielsen, H. Nilsen, Trends Food Sci. Technol. 8 (1997) 258–265.
[7] N. Hamada-Sato, K. Usui, T. Kobayashi, C. Imada, E. Watanabe, Food Control 16 (2005) 301–307.
[8] L. Gram, H.H. Huss, Int. J. Food Microbiol. 33 (1996) 121–137.
[9] K. Crowley, A. Pacquit, J. Hayes, K.T. Lau, D. Diamond, in: Proc. IEEE Sensors, 2005, pp. 754–757.
[10] A. Pacquit, K.T. Lau, D. Diamond, in: Proc. IEEE Sensors, 2004, pp. 365–367.
[11] B. Kuswandi, Jayus, A. Restyana, A. Abdullah, L.Y. Heng, M. Ahmad, Food Control 25 (2012) 184–189.
[12] Z. Jun, L.X. Yu, W. Wei, Z. Zhu, Z. Wei, X. Wu, in: Proc. IEEE World Congress on Computer Science and Information Engineering, 2009, pp. 221–224.
[13] X. Li, Electronic Nose to monitor the freshness of Red Fish (*Sebastes marinus*) stored in ice and modified atmosphere packaging (MAP), UNU-Fisheries Training Programme, Final Project 2000, Reykjavik, Iceland, 2000.
[14] J. Hammond, B. Marquis, R. Michaels, B. Oickle, B. Segee, J. Vetelino, A. Bushway, M. E. Camire, K. Davis-Dentici, Sens. Actuators B: Chem. 84 (2002) 113–122.
[15] L. Zhongdong, G. Benqian, H. Yaqiang, L. Zhenxing, L. Boxiang, in: Proc. IEEE Complex Medical Engineering, 2011, pp. 165-168.
[16] S. Bhadra, D.J. Thomson, G.E. Bridges, Sens. Actuators B: Chem. (2014), http://dx.doi.org/10.1016/j.snb.2014.12.021.
[17] B.E. Horton, S. Schweitzer, A.J. DeRouin, K.G. Ong, IEEE Sens. J 11 (2011) 1061–1066.
[18] G.S. Duffó, S.B. Farina, C.M. Giordano, Electrochim. Acta 54 (2009) 1010–1020.
[19] S. Bhadra, M. McDonald, D.J. Thomson, M.S. Freund, G.E. Bridges, in: Proc. IEEE RFID, 2014, pp. 147–152.
[20] S. Bhadra, D.S.Y. Tan, D.J. Thomson, M.S. Freund, G.E. Bridges, IEEE Sens. J 13 (2013) 2428–2436.
[21] J. Crank, Methods of measurement, in: J. Crank, G.S. Park (Eds.), Diffusion in Polymers, Academic Press, New York, 1968, pp. 1–39.
[22] S. Neethirajan, M.S. Freund, D.S. Jayas, C. Shafai, D.J. Thomson, N.D.G. White, Biosyst. Eng. 106 (2010) 395–404.
[23] Frozen fish. ⟨http://www.tis-gdv.de/tis_e/ware/fisch/gefroren/gefroren.htm⟩, 2014 (accessed 14.08.14).
[24] K. Koutsoumanis, Appl. Environ. Microbiol. 67 (2001) 1821–1829.
[25] S. Khan, R. Dahiya, L. Lorenzelli, IEEE Sens. J. PP (2014), http://dx.doi.org/10.1109/JSEN.2014.2375203.
[26] T. Ge, J.S. Chang, T. Lin, Z. Lei, L.G. Soon, in: Proc. IEEE International Midwest Symposium on Circuits and Systems, 2014, pp. 825–828.
[27] P. Majzlikova, J. Prasek, J. Chomoucka, J. Drbohlavova, J. Pekarek, R. Hrdy, J. Hubalek, L. Trnkova, in: Proc. IEEE Sensors, 2013, pp. 1–4.
[28] P. Kurzweil, Sensors 9 (2009) 4955–4985.
[29] INTRASITE Gel. ⟨http://www.smith-nephew.com/professional/products/advanced-wound-management/intrasite-gel/⟩, 2013 (accessed 27.07.13).

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