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

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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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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.

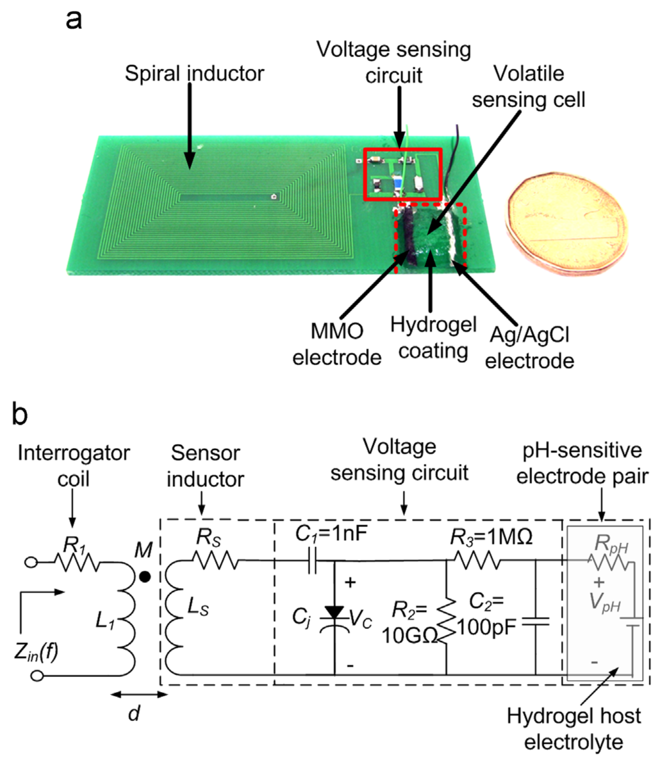

Fig. 1. (a) Prototype volatile absorption sensor with hydrogel coated MMO and Ag/AgCl electrodes. The prototype is fairly large and can be further miniaturized. (b) Equivalent circuit diagram of the passive sensor including the near-field coupled interrogator coil (interrogator not shown in Fig. 1a).

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 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

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