Simple and Low-Cost On-Package Sticker Sensor based on Litmus Paper for Real-Time Monitoring of Beef Freshness

Bambang Kuswandi[1]*, Fitria Damayanti[1], Jayus[1], Aminah Abdullah[2] & Lee Yook Heng[2]

[1] Chemo and Biosensors Group, Faculty of Pharmacy, University of Jember
Jalan Kalimantan 37, Jember, 68121, Indonesia
[2]School of Chemical Sciences & Food Technology, Faculty of Science & Technology,
43600 UKM, Bangi Selangor, Malaysia
*Email: b_kuswandi.farmasi@unej.ac.id

Abstract. A simple sticker sensor has been constructed using litmus paper and tests have been conducted to detect the freshness of beef samples. The results show that the sticker sensor can be used to determine the degree of beef freshness, since the color change of the litmus paper and the quality degradation of the beef during storage time had a similar trend, where the decay of the beef could be detected clearly (when the red litmus paper changed to blue). The sticker sensor reacted accurately to the beef's freshness in terms of pH change due to beef deterioration from pH 5.61 to 6.24 and from pH 5.67 to 6.02 as shown by its color change in real time at room and chiller temperature respectively. Thus, the sticker sensor can be used as an effective tool for monitoring the microbial quality of packaged fresh meat that correlates with the increased pH of the beef, where the total viable count (TVC) of 5 x 10^6 cfu/g or 6.698 log cfu/g correlates with a pH of 6.24. These levels were reached at 10 hrs and 7 days at room and chiller temperature respectively. This study provides a foundation for developing a simple sensor for beef freshness.

Keywords: *beef freshness; litmus paper; meat packaging; pH; sticker sensor.*

1 Introduction

Since the last decade there have been many serious incidents concerning food safety. For example, at the beginning of 2001, meat was infected with *Listeria* in France, and in 2006, chloromycetin was detected in the products of shell fish from Asia that had been exported to Europe, America and Canada [1]. Great attention is paid to food safety around the world, due to many people suffering from food poisoning every year. For instance, the chemical compounds of meat are very complex. There is about 10-30% fat, 10-20% protein and 1-5% sugar in meat [2]. The degradation speed of meat is high because microorganisms can easily develop in it. According to the present quality criterions for beef, there are three kinds of methods to evaluate the quality of beef, i.e. sensory [3], chemical [4] and microbiological methods [5].

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Sensory evaluation is a subjective method where the results depend on the skills of the operator, which causes errors. The odor of meat for example, gives it a number of unique qualities and characteristics. However, it is difficult to correlate with sensory evaluation. Chemical methods for inspecting meat quality are: total volatile basic nitrogen (TVBN), pH and triphenyltetrazolium chloride (TTC) determination [6]. Microbial methods for evaluating meat quality are: total viable count (TVC), brochothrix thermosphacta, lactic acid bacteria (LAB), Pseudomonas spp, and enterobacteriaceae determination [6,7,8]. However, these methods have shortcomings, such as having lengthy procedures, being time-consuming, expensive and insufficiently precise. Gas measurement is one of the chemical methods for quality evaluation of meat. Gas chromatography is commonly used for measuring gas contents and can be used to measure many chemical compounds compared with standard chemicals [9]. However, this method is only appropriate for research and laboratory conditions. Therefore, a simple, low-cost, highly efficient and effective method is needed to evaluate the quality of meat.

Color based pH indicators offer potential for use as indicators of the microbial metabolites for freshness monitoring. This method can be used for on-package monitoring of food spoilage. For instance, an immobilized pH sensitive dye (bromocresol green) has been proposed as fish spoilage indicator or sensor [10, 11]. This sensor works based on pH, which spoilage gradually produces, basic volatile amines in the food package headspace, which causes the pH to increase and subsequently the color of the sensor will change from yellow to blue, easily visible to the naked eye. Works using the same principle have been reported in by Kuswandi, *et al.* [12-15]. To arrive at a simpler and more practical approach using the same principle, we applied litmus paper as on-package sticker sensor. Litmus paper can easily be prepared: it is commercially available worldwide, low-cost and safe if in contact with meat. The ability of litmus paper to change color when exposed to an acid or base is the result of litmus paper being infused with lichens (fungi/natural dye).

The purpose of this study was to use litmus paper to construct a simple and low-cost on-package sticker sensor for determining the freshness of beef. Litmus paper is known as a material highly sensitive toward acid-base reactions. Color changes (from red to blue for spoilage indication) as a result of its interactions with pH due to an increase in basic spoilage volatile amines, were monitored directly with a colorimeter. The membrane response was found to correlate with sensory evaluation, pH, TVBN and bacterial growth patterns in beef samples. The performance of this sticker sensor was successfully tested directly by real-time monitoring of beef freshness in ambient and chiller conditions.

2 Materials and Methods

2.1 Preparation of Beef Samples

Fresh tenderloin beef of normal pH (5.6-5.7) purchased at a local butcher shop in Jember was used in this study. Other parameters that affect the rate of beef quality deterioration were kept relatively the same, e.g. the specific muscle used was tenderloin, relative postmortem time when purchased was 5 hrs, the diet of the cattle was from the same source, and the packaging and transport from the butcher shop to the lab was done in the same way using styrofoam as container with a transport time of around 30 mins. The meat was divided into portions of 100 g and 50 g for microbiological and sensory analysis, respectively, placed on plastic trays and enclosed into low-density polyethylene plastic film (Carrefour, Indonesia). The samples were stored at chiller conditions (4 ± 0.2°C) in a low-temperature incubator (model MIR 153, Sanyo Electric Co., Japan) and at room temperature (28 ± 2°C). The temperature of the samples was monitored throughout the entire storage period using electronic temperature recording devices (Cox Tracer, Belmont, NC). Triplicate packages of the meat product, from each storage temperature, were sampled at appropriate time intervals to allow for efficient kinetic analysis of microbial growth, pH measurement and sensory evaluation of color and odor for the study of microbial spoilage of the meat stored under chiller or room storage conditions. All experiments were conducted three times.

2.2 Microbiological Analysis

Samples (25 g) of meat were aseptically weighed, added to 1/4 strength Ringer's solution (225 ml), and homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature. Decimal serial dilutions in ¼ strength Ringer's solution were prepared and duplicate 1 ml or 0.1 ml samples of appropriate dilutions were poured or spread on the surface of the appropriate media in petri dishes for enumeration of (i) total aerobic viable count (TVC) on plate count agar (PCA; Merck, Darmstadt, Germany), incubated at 25°C for 72 hrs, and (ii) Pseudomonas spp. on cetrimide-fucidin-cephaloridine agar (CFC Oxoid, CM559 supplemented with selective supplement SR 103E, Basingstoke, UK) incubated at 25 °C for 48 hrs. Both plates were examined visually for typical colony types and morphological characteristics that were associated with each growth medium. In addition, the selectivity of each medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies obtained from both media.

2.3 Measurement of pH and Volatile Amine in Beef Samples

The pH values were recorded by a pH meter (Russel, Moder RL150) with the glass electrode being immersed in the homogenate of the meat after the end of the microbiological analysis. Perchloric acid (PCA) extract of the beef samples was prepared and analyzed for TVBN levels according to Pearson [16]. All the beef samples were washed thoroughly with tap water. The beef was skinned aseptically on one side and minced by passing three times through a meat grinder with 4 mm holes. 10 g of beef sample were blended with 90 ml of PCA 6%. 50 ml of filtrate was made alkaline with hydroxide 20% and distilled 10 min in a 2100 Kjeltec Distillation Unit (FOSS Tecator AB). Each analysis was repeated three times.

2.4 Sensory Analysis

Sensory evaluation of the beef samples was performed during storage at chiller or room temperature by a five-member panel composed of staff from the laboratory. The panelists were trained to objectively evaluate the samples to give similar responses to the same sample characteristics. The same persons were used for each evaluation session and all were blinded to the age and temperature history of the product being tested. The sensory evaluation was carried out under artificial light and the temperature of the packed product approximated the ambient or room temperature. Special attention was given to color, texture and odor. The texture of the meat was measured using a texture meter (Rheotex, UK). Odor was judged and recorded in appropriate form with descriptive terms, reflecting the organoleptic evolution of quality deterioration [17], for which a simple three-point scoring system was adapted [16,18]. Each attribute was scored on a continuous 0 to 3 hedonic scale with 0 being the highest quality score, 1 given to an acceptable product, 2 being the limit of product acceptance or rejection point, and 3 being an unacceptable meat sample.

2.5 Measurement of the Sticker Sensor Response

The sticker sensor consisted of red litmus paper (Merck, UK), designed as shown in Figure 1. In order to evaluate the applicability of the developed sticker sensor to monitor the spoilage process of meat, the sticker sensor was placed inside the package of the beef samples, where the litmus paper was in direct contact with the atmosphere inside the package, and stored at chiller and room temperature. This method was used to make sure that there would be no effect from external atmospheric conditions. For the control, the sticker sensor was placed inside a package without beef sample.

The distinct, irreversible color change of the sensor from the initial red to the final blue (end point of red litmus paper) was used as the measurable response of change. The kinetics of color change of the sensor system were assessed using a hand-held colorimeter (chroma meter CR-10, Minolta Inc., Japan) to determine the CIE color space co-ordinates, i.e. color visible to the human eye, as specified by the International Commission on Illumination (*Commission Internationale d'Eclairage, CIE*), L*, a*, b*, and c*. CIE L* (lightness), a* (redness), and b* (yellowness) values, and c*, Chroma (also referred to as saturation index and color intensity) were calculated as: $[(a^{*2}+b^{*2})^{0.5}]$. Here, for simple measurement c* (color intensity) was used as sensor response for the intensity of the red color of the litmus paper (in arbitrary units) in all experiments.

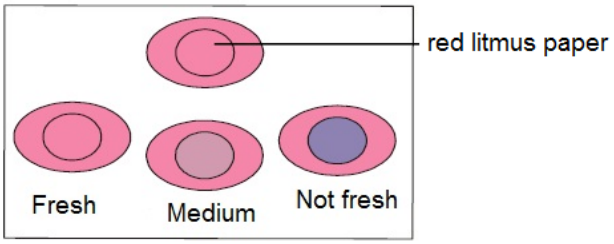


Figure 1 Design of sticker sensor based on red litmus paper for beef freshness monitoring with color indication for fresh, medium/still fresh (needs to be consumed within hours) and not fresh (spoilage, do not consume).

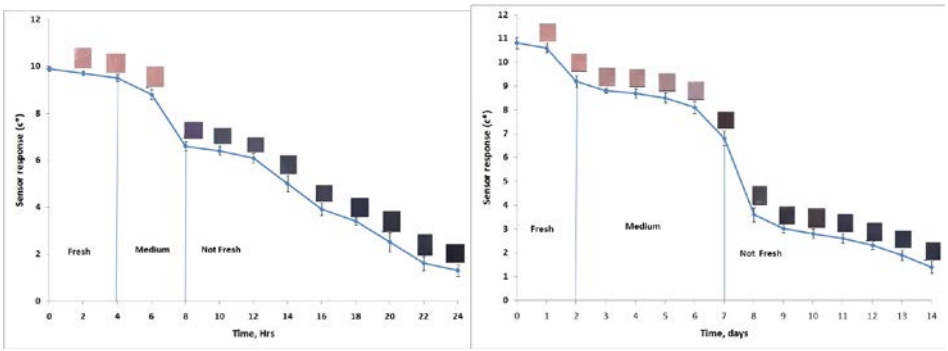
3 Results and Discussion

3.1 Response of Sensor toward Beef Spoilage

All sensors were placed in close proximity to the meat samples in order for them to respond to the increasing volatile amines generated by spoilage with a very distinct color change from red to blue. The sensors were monitored periodically until no further color change was observed. Figures 2A and 2B show the rate of color change of the sensors (c*, intensity of red in arbitrary unit) towards spoiling meat at room and chiller temperatures. In Figure 2A, the sensor response's steady decrease (as the red litmus color changes to blue) within 24 hrs of the experiment was observed at room temperature. Here, the sensor gradually changed color from red to blue at 8 hrs at room temperature. While in chiller temperature as shown in Figure 2B, prior to the first 3 days no drastic color change was observed. Then at day 7, the litmus changed to blue. In general, the red litmus changed color from red to purple after 3 days and then to blue at day 7 at chiller temperature. Furthermore, visual inspection did not detect differences in color between the sensors of different batch samples. The

onset of spoilage was detected at 8 hrs and 7 days for room and chiller temperatures respectively. This indicates that the beef samples released volatile amines at a relatively slow rate, since its freshness lasted for 8 hrs and 7 days for room and chiller temperatures respectively.

False positives did not occur with the proposed sticker sensor, since the sensor was placed inside the plastic cover, so that there was no contact with the ambient environment, only direct contact with the atmosphere inside the package for headspace detection and monitoring of beef freshness. However, a false negative could occur if the plastic cover of the beef package is broken, which would result in basic spoilage volatile amines leaking out, reducing the concentration of volatile amines inside the package, which in turn would cause an error in the sensor's response. To reduce false positives one only has to make sure that the package is in the best condition and the sticker sensor placed in a correct position.



(a) (b)

Figure 2 Rate of color changes of red litmus paper as sensor response (c*) towards spoiling beef at room (a) and chiller (b) temperature.

The precision of the sensor response related to the reproducibility of the measurement is shown as the error bars in Figure 2, where error values were smaller than 5%, which is acceptable for this type of measurement [19]. Furthermore, as for the ruggedness or robustness [19,20] of the sticker sensors, they were prepared before they were used in different batches on different days to test the sensor response. Based on our experiment, the sensor showed a consistent response toward beef freshness.

3.2 pH and TVBN Analysis of Beef Samples

Figure 3(a) and 3(b) show the pH values of the beef samples along with the sensor response. At room temperature, the pH values of the beef sample varied

from pH 5.61 at the fresh stage to pH 6.24 at the spoilage stage at 8 hrs (Figure 3(a)). At chiller temperature, the pH values of the beef samples varied from pH 5.67 at day 1 to pH 6.02 at day 7. It can be seen from both figures (Figures 3(a) and 3(b)) that the sensor response follows a similar trend as shown by the pH response under both conditions. Furthermore, the sensor also responded to the increase in pH value in the package headspace, since the range of the red litmus paper's color change is related to the pH levels in the beef sample.

According to Dainty [4] meat spoilage occurs at high pH (> 6.0), at lower cell densities than at normal pH (< 5.8) of fresh meat. This value was reached at 8 hrs and 7 days of storage at room and chiller temperature respectively. Thus, the sensor gave an accurate response, since the indication of spoilage was also given at 8 hrs and 7 days for room and chiller temperature respectively.

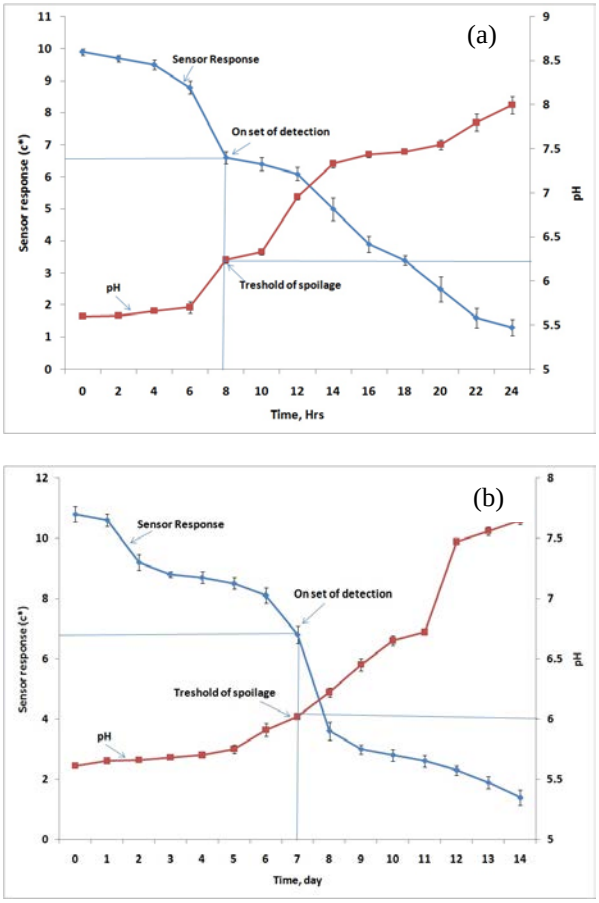


Figure 3 pH values of beef samples and sensor response at room (a) and chiller (b) temperature.

Volatile basic amine (TVBN) levels rose due to formation of NH_3 and other volatile amines. Biogenic amines such as histamine, putrescine, tyramine and cadaverine have been implicated as amine indicators of meat product decomposition [21,22]. The concentration of produced ammonia has been found to be proportional to the concentration of biogenic amines and can hence be used for the determination of biogenic amines in meat matrixes as well [23].

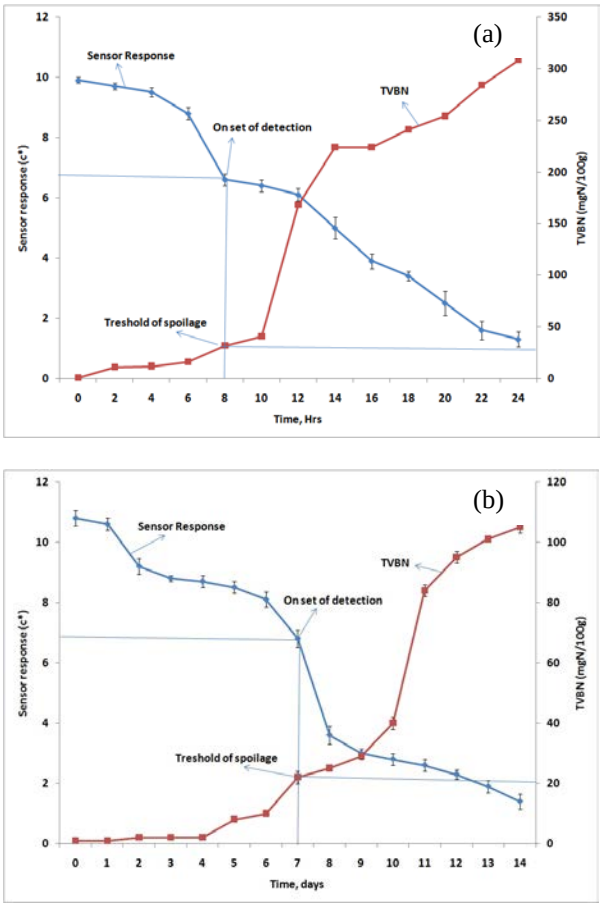


Figure 4 TVBN values of beef samples and sensor response at room (a) and chiller (b) temperature.

The results for this measurement are given in Figures 4(a) and 4(b) along with the sensor response at room and chiller temperature respectively. It can be seen that the sensor response followed a similar trend as shown by TVBN determination. Furthermore, the sensor accurately responded to the increase in volatile base concentration in the package headspace, since the range of sensor

color change is related to the levels of TVBN in the beef samples. Along with the decrease of freshness, the TVBN increases. The TVBN value for hygienic-standard meat of livestock is ≤ 20 mg/100 g [24]. These levels were reached at 8 hrs and 7 days at room and chiller condition respectively, which is similar with the sensor response as given in both Figures 4A and 4B, where the sensor indicates that the packaged beef showed spoilage or deterioration at 8 hrs and 7 days at room and chiller temperatures respectively.

3.3 Sensory Analysis of Beef Samples

The color, texture and odor of the beef samples were first evaluated by sensory evaluation and the results were recorded. The measurements were conducted along with the sensor response and these results were also recorded. The results

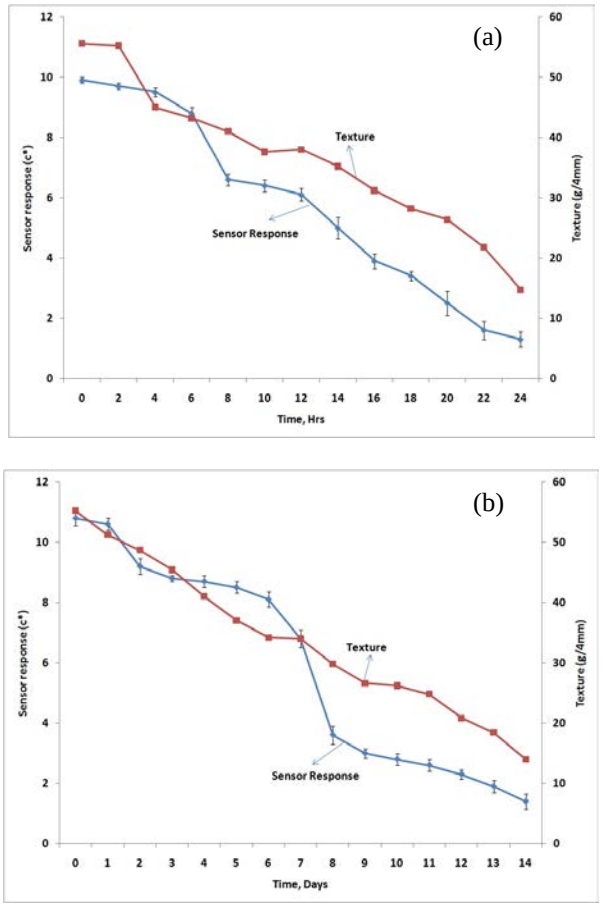


Figure 5 Texture values of beef samples and sensor response at room (a) and chiller (b) temperature.

of the sensor response were confirmed by the sensory evaluation. The measurements were done under laboratory conditions without any special requirements considering the prospective application at shopping centers, restaurants, storage rooms and others. Figures 5(a) and 5(b) are the average values of the texture readings from the Rheotex used for the measurement of the beef samples at room and chiller temperatures respectively. Each datum is the average of three measurements under identical conditions. It can be seen from both figures (Figures 5(a) and 5(b)) that the sensor response showed a trend similar to the texture value. The freshness decreased along with the decreasing texture value of the beef samples.

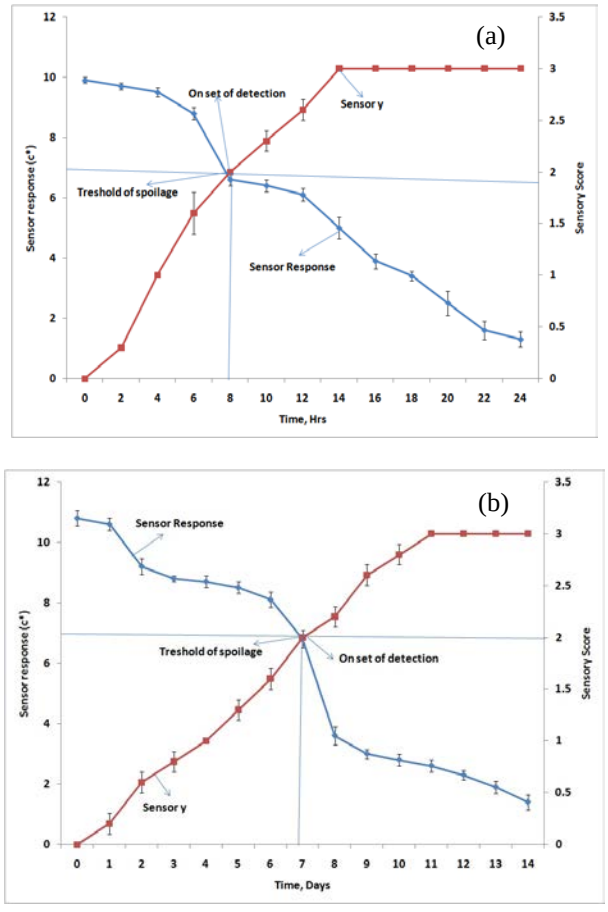


Figure 6 Sensory score of beef samples and sensor response at room (a) and chiller (b) temperature.

Figures 6(a) and 6(b) show the output score of the odor measurements corresponding to Tables 1 and 2. Tables 1 and 2 list the results of the sensory evaluation at room and chiller temperature respectively. From both figures, it can be seen that the sensor response showed a similar response to the sensory response (score), where the point of rejection of the sensory score (2) was similar to the onset of detection of the sensor response. This is indicated by the color change of the litmus paper from red to blue for spoilage indication as given in Tables 1 and 2 for room and chiller temperatures respectively.

Table 1 Results of sensory evaluation of beef samples at room temperature

Stored time (hrs)	Color	Odor	Color of sticker sensor
0	Fresh red	No peculiar smell	Red
2	Fresh red	No peculiar smell	Red
4	Deep red	No peculiar smell	Red
6	Dark red	No peculiar smell	Purple-red
8	Black red	Light peculiar smell	Blue
10	Black	Smelly	Blue
12	Deep black	Stink	Blue
14	Dark black	Foul	Dark blue
16	Dark black	Foul	Dark blue
18	Dark black	Foul	Dark blue
20	Dark black	Foul	Dark blue
22	Dark black	Foul	Dark blue
24	Dark black	Foul	Dark blue

Table 2 Results of sensory evaluation of beef sample at chiller temperature.

Stored time (day)	Color	Odor	Color of sticker sensor
0	Fresh red	No peculiar smell	Red
1	Fresh red	No peculiar smell	Red
2	Fresh red	No peculiar smell	Red
3	Fresh red	No peculiar smell	Red
4	Deep red	No peculiar smell	Red
5	Deep red	No peculiar smell	Red
6	Dark red	No peculiar smell	Purple-red
7	Black red	Light peculiar smell	Blue
8	Black red	Light peculiar smell	Blue
9	Black	Smelly	Blue
10	Deep black	Stink	Blue
11	Dark black	Foul	Dark blue
12	Dark black	Foul	Dark blue
13	Dark black	Foul	Dark blue
14	Dark black	Foul	Dark blue

3.4 Microbial Analysis of Beef Samples

The TVC counts steadily increased from 2.71 x 10^3 cfu/g during the initial 2 hrs to 8.1 x 10^5 cfu/g at 10 hrs of investigation at room temperature (Figure 7(a)). Initially, the *pseudomonas* counts were at approximately 70% of the TVC counts rising to approximately 80% at 24 hrs at room temperature. Here, the *pseudomonas* counts increased sharply until 16 hrs of investigation with ca 3.8 x 10^7 cfu/g at room temperature. At chiller temperature, initially the TVC counts were at 1.2 x 10^3 cfu/g at day 1 and rising to 1.1 x 10^5 cfu/g at day 7 at chiller temperature (Figure 7(b)). The *pseudomonas* counts were at approximately 50% of the TVC counts rising to approximately 80% at day 7 at chiller condition. Then, they increased steadily, similar to the TVC count at chiller condition. When compared to the sensor response in both figures, it can clearly be seen

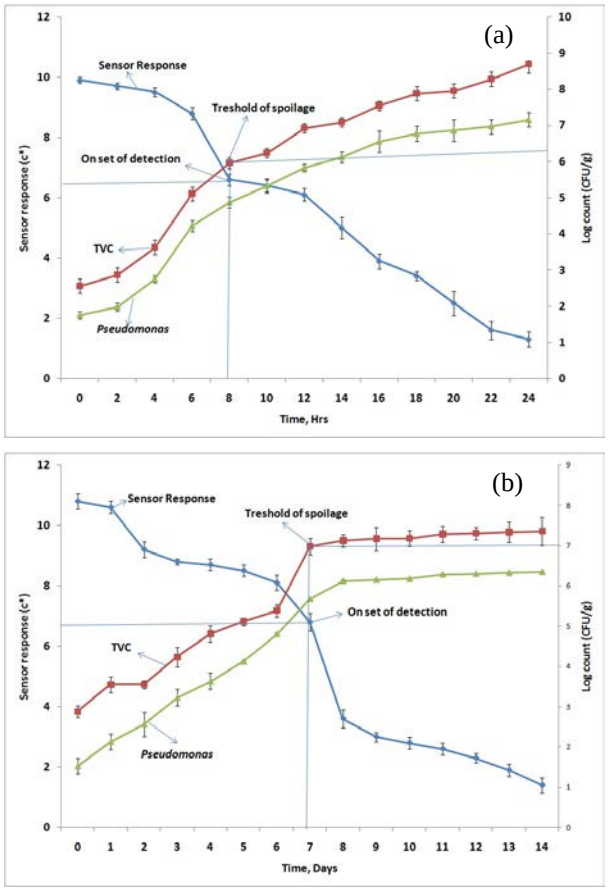


Figure 7 TVC and *pseudomonas* count of beef samples and sensor response at room and chiller temperature.

that not only does the sensor response correlate with the changes in bacterial populations but the sensor color change from orange to reddish orange also correlates with the level of product rejection (5×10^6 cfu/g or 6.698 log cfu/g) according to the TVC value used in Indonesia for meat products [25]. These levels were reached at 10 hrs and 7 days at ambient and chiller condition respectively. Thus, the sensor gave an accurate response to the increase in volatile base concentration in the package headspace, particularly at chiller temperature. However, at room temperature, the sensor gave an earlier response as onset of detection (8 hrs) toward the threshold of microbial detection (Figure 7(a)). This means that false positives may occur in this case, if the sensor's color already changes before the TVBN levels in the headspace rise. In general, the range of the sensor color change can be related to the higher levels of microbial population in the beef sample. In addition, the visual color changes of on-package sensors are useful indicators of the approximate microbial population and therefore spoilage of the beef samples (Figure 8). Finally, it can be clearly stated that this sensor can be used to indicate the presence of high microbial populations in packaged beef, the color of the sensor changing to blue for visual identification that the beef is spoiled and cannot be consumed anymore.

Figure 8 Application of red litmus paper as a sticker sensor for beef freshness monitoring at 4 hrs (left) and 24 hrs (right) at room temperature.

4 Conclusions

Litmus paper was used to construct a simple sticker sensor for monitoring beef freshness. The relationship between sensor response and beef freshness was investigated. The test results show that the sensor can be used for detecting beef freshness, since there was a correlation between the color change of the litmus paper as a sensor response and the quality degradation of the beef during storage time, where the decay of the beef could be detected clearly (the red color changing to blue). The sensor responds to fresh beef with a red color and

displays an intense color change to blue in response to decayed beef. The sticker sensor reacted accurately to beef freshness in terms of its pH change as shown by its reliability and sensitivity of detection in real time. Thus, the sticker sensor can be used as an effective tool for monitoring the microbial quality of packaged fresh meat. The sticker sensor may serve as an active shelf-life labeling device in conjunction with the "used-by-date" labeling, when attached to individual product units, or may be used to optimize distribution control and management of a stock rotation system, thereby reducing food waste.

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