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A Novel On-Package Sticker Sensor Based on Methyl Red for Real-Time Monitoring of Broiler Chicken Cut Freshness

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ABSTRACT

A novel sticker sensor has been fabricated based on methyl red, and tests have been conducted to detect the freshness of broiler chicken cuts. Methyl red was immobilized onto a bacterial cellulose membrane via absorption method. The methyl red/cellulose membrane as a freshness sensor worked based on pH increase as the basic spoilage volatile amines produced gradually in the package headspace, and subsequently, the colour of the sensor will change from red to yellow for spoilage indication, which is easily visible to the naked eye. The results show that the sticker sensor could be used to determine the degree of chicken cut freshness, as the relationship between the colour change of methyl red as a sensor response and the chicken cut freshness follows a similar trend. Therefore, the spoilage of the chicken cut could be detected visually. A sticker sensor indicates the chicken cut freshness by its colour change in real time. Thus, the sticker sensor can be used as an effective tool for monitoring the microbial quality of packaged fresh poultry meat. Finally, the methyl red/cellulose membrane was successfully used as a sticker sensor for the real-time monitoring of chicken cut freshness in ambient and chiller conditions. Copyright © 2013 John Wiley & Sons, Ltd.

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KEY WORDS: sticker sensor; methyl red; bacterial cellulose; chicken cuts; freshness; meat packaging

INTRODUCTION

Currently, the growing demand of consumers for the freshness and safety of food products is increasing continually. In order to meet these needs, new packaging technologies that have intelligent functions and identification methods in packaging, such as smart packaging, have been developed.[1–4] For instance, the integrity of the package and the time–temperature (TT) indicator during the storage of the product have a great impact on the quality of the packaged food. A smart packaging with an intelligent indicator, which gives information on the integrity and the TT history of the food package, would be really helpful in assuring the quality and safety of the packaged food product.[5,6] Nowadays, TT indicators are already commercially available, and their use is increasing in the food industry, particularly for perishable foods.[7–10]

Chicken is a highly perishable food, since it usually deteriorates within a week of slaughtering, regardless of storage chiller systems. Such spoilage is mainly due to different types of microorganisms, e.g. *Pseudomonas* spp., *Shewanella putrefaciens* and yeasts, depending on the initial microbiological quality of the poultry carcasses.[11] Therefore, reliable methods for assessing the microbiological quality and/or freshness of meat would benefit both the consumers and the meat industry. Shelf-life

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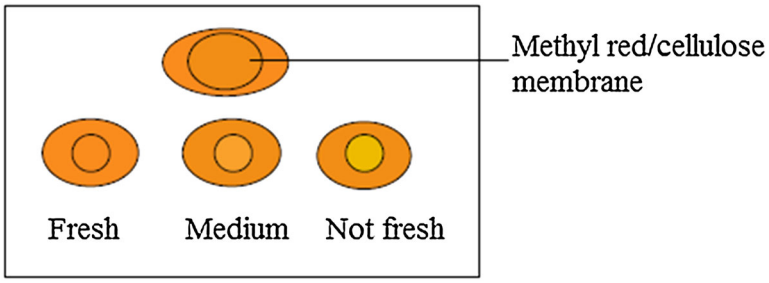

Figure 1. The design of the sticker sensor based on methyl red/cellulose membrane for broiler chicken cuts' freshness with colour indication for fresh, medium (need to be consumed in hours) and not fresh (spoilage, do not consume).

studies of perishable meat and meat products are usually carried out by evaluating the microbiological and sensory quality of the product as a function of storage time. Because these microbial analyses are costly and time consuming, alternative methods, involving chemical changes due to microbial growth, have also been suggested as quality indicators of meat.[12]

Biogenic amines (BAs) are known to be generated by the growth of decarboxylase-positive microorganisms under conditions favourable to enzyme activity.[13] Numerous bacteria have been reported to possess amino acid–decarboxylase activity.[14] Many *Enterobacteriaceae*, *Pseudomonas* spp. and certain lactobacilli, enterococci and staphylococci are particularly active in the formation of BAs. The amount of amines formed depends abundantly on the type of microorganisms present.[15] Therefore, the formation of amines, including BAs as non-volatile amines, and volatile amines (VAs), such as trimethylamine (TMA) and total volatile basic nitrogen (TVBN), is primarily a consequence of the enzymic decarboxylation of specific amino acids due to microbial enzyme activity.[16] Although the determination of BAs in poultry meat has been proposed as a useful indicator of spoilage,[13,17,18] there is little information on the chemical changes of VAs occurring in meat,[19,20] including poultry meat during storage.

There are several studies on the possible use of BAs in determination of meat quality. Silva and Gloria[19] reported levels of BAs in chicken meat and chicken-based meat products. Immediately after slaughter, spermine and spermidine were detected in breast and thigh meat. The presence of putrescine, cadaverine, histamine and tyramine was observed in chicken samples after 15 days of refrigerated storage.

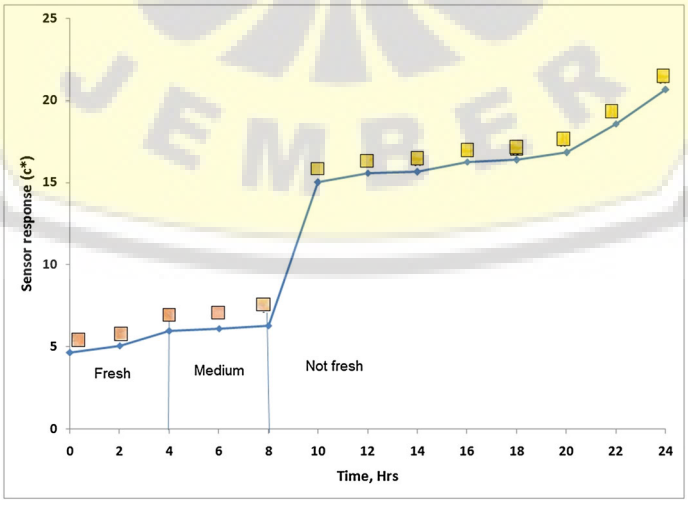

Figure 2. The colour changes of methyl red/cellulose membrane as a sensor response (c*) versus time of chicken cuts stored at room temperature ($n = 4$); sensor colours are shown in the boxes above the line.

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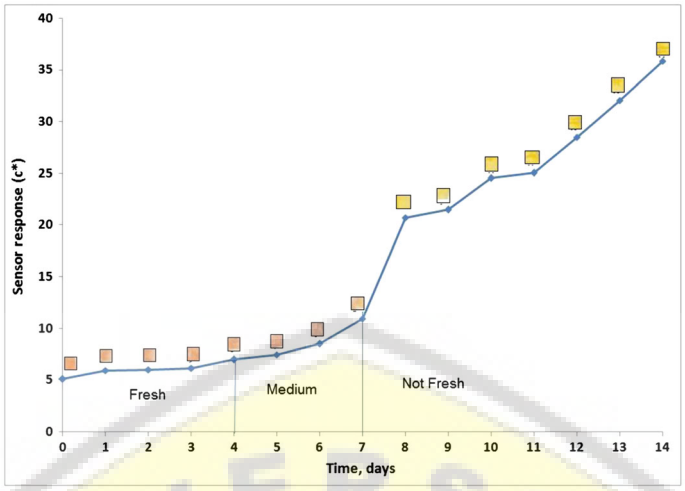

Figure 3. The colour changes of methyl red/cellulose membrane as a sensor response (c*) versus time of chicken cuts stored at chiller temperature ($n = 4$); sensor colours are shown in the boxes above the line.

Balamatsia *et al.*[17] also reported the formation of BAs in breast chicken meat during storage under aerobic and modified atmospheric packaged (MAP) conditions at 4°C. Levels of putrescine and cadaverine increased linearly with storage time and were higher in aerobically packaged chicken samples. Levels of tyramine in chicken samples stored aerobically and/or under MAP conditions were low (<10 mg/kg), whereas spermine and spermidine levels were also detected in both aerobically stored and MAP-stored chicken meat. It was also concluded that tyramine, putrescine and cadaverine could serve as quality indicators of MAP broiler chicken meat.[13] With regard to meat quality, only recently, TVBN limit values of approximately 20 and 30 mg N per 100 g for beef and pork (corresponding to 8 and 10 days of refrigerated storage, respectively) have been proposed as indicators of meat quality.[21] However, BA and VA determination usually involves high-performance liquid chromatography, which needs sample destruction and preparation, laborious extraction and clean-up. This is time consuming and not suitable for field application and on-line monitoring. By using TVBN value as VAs for quality indicator of packaged chicken, at the term of its contribution to increase the pH inside the atmospheric food packaging during storage, a simple method could be developed based on the pH indicator in this range (pH 5.0–6.0).

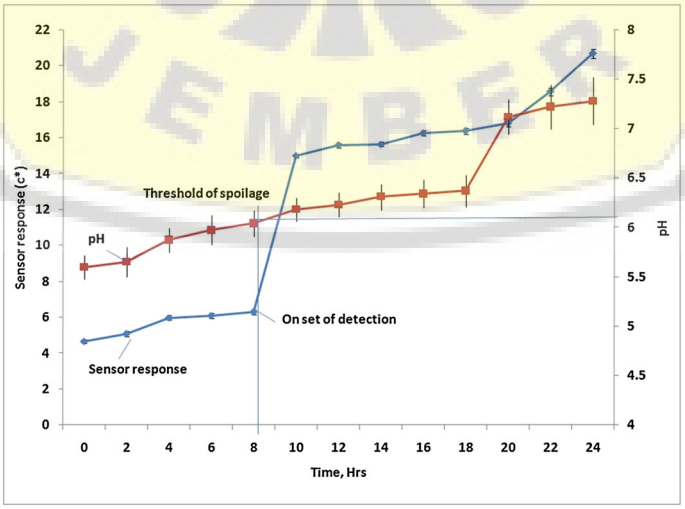

Figure 4. The pH values of chicken meat samples and the sensor response at room temperature ($n = 4$).

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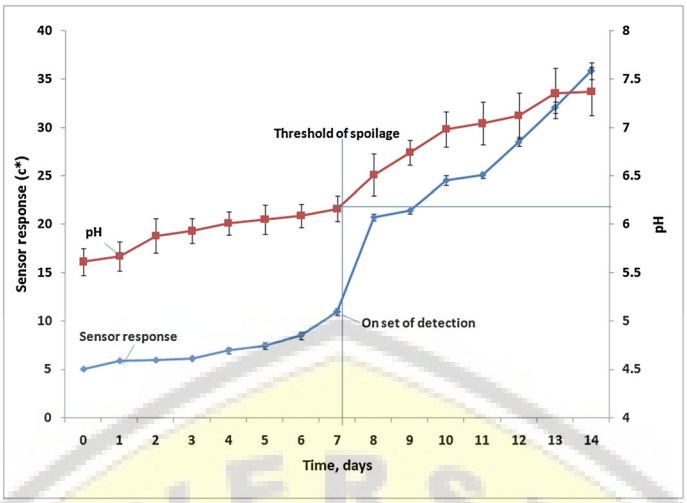

Figure 5. The pH values of chicken meat samples and the sensor response at chiller temperature ($n = 4$).

The number of publications on package indicators for spoilage or freshness of food is still limited. However, there are some attempts to construct indicators for the volatile compounds produced in microbial spoilage with the use of a pH indicator,[21,22] including polyaniline [23] and curcumin.[24] This sensor worked based on pH, as the basic VAs produced gradually in the spoilage food package headspace caused the pH increase, and subsequently, the colour of the sensor will change into something that is easily visible to the naked eye. Based on a similar principle, by using indicator dyes such as methyl red (pK_a of 4.80), it could also be used as a freshness indicator for poultry meat, e.g. broiler chicken meat. Since fresh chicken meats have a pH of around 5.50 and a pH above 6.0 for spoilage chicken meat,[12, 25] the increase of pH could occur during deterioration of meat products, due to the increase in VAs.[26]

The purpose of this study is to use methyl red/cellulose membrane to construct a low-cost, on-package sticker sensor for determining the freshness of packaged chicken cuts. The methyl red/cellulose membrane is a highly sensitive material with a pH towards the range of 5.0–6.0. Colour changes (from

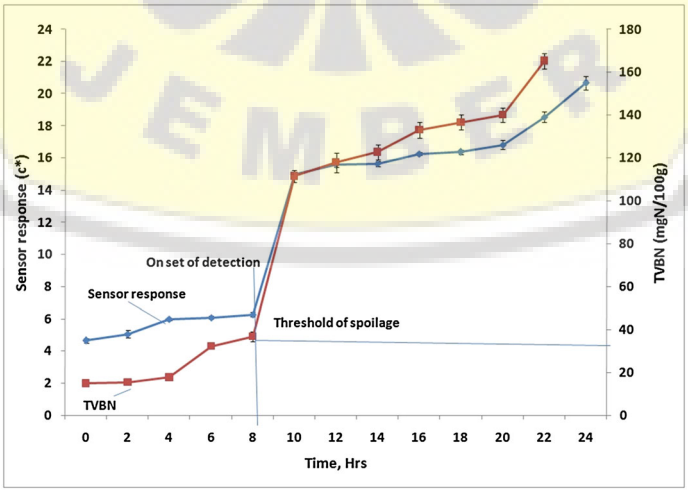

Figure 6. The TVBN values of chicken meat samples and the sensor response at room temperature ($n = 4$).

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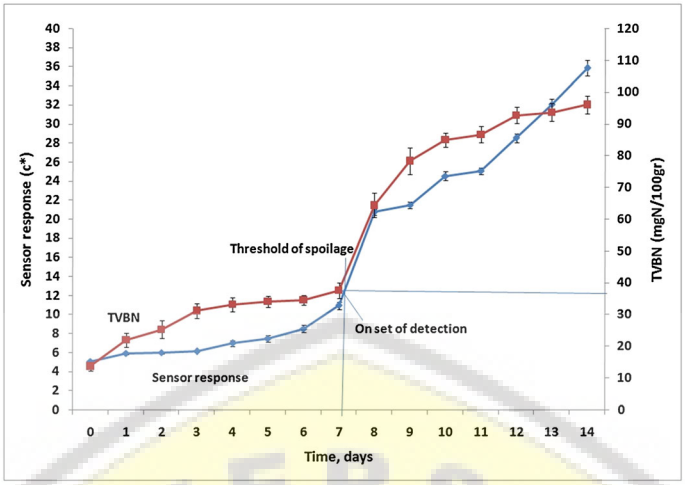

Figure 7. The TVBN values of chicken meat samples and the sensor response at chiller temperature ($n = 4$).

red to yellow for spoilage indication), as a result of its interactions with pH due to an increase in VAs, were monitored directly using a colorimeter. Furthermore, the membrane response was found to correlate with pH, TVBN, sensory evaluation and bacterial growth patterns in chicken cut samples. The performance of this sticker sensor was successfully tested directly for real-time monitoring of packaged chicken cuts' freshness in ambient and chiller conditions.

MATERIALS AND METHODS

Chemicals

A stock solution of methyl red (Sigma, UK) was prepared by dissolving 10 mg of methyl red in 10 ml of ethanol (50%) to give a concentration of 1 mg/ml. All chemicals used were of reagent grade (supplied by Merck, Darmstadt, Germany; Sigma; or Fluka, UK) and used as supplied.

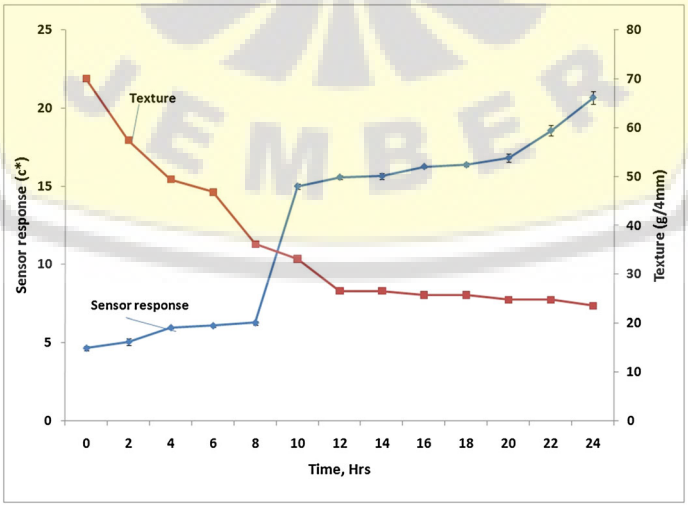

Figure 8. The texture values of chicken meat samples and the sensor response at room temperature ($n = 4$).

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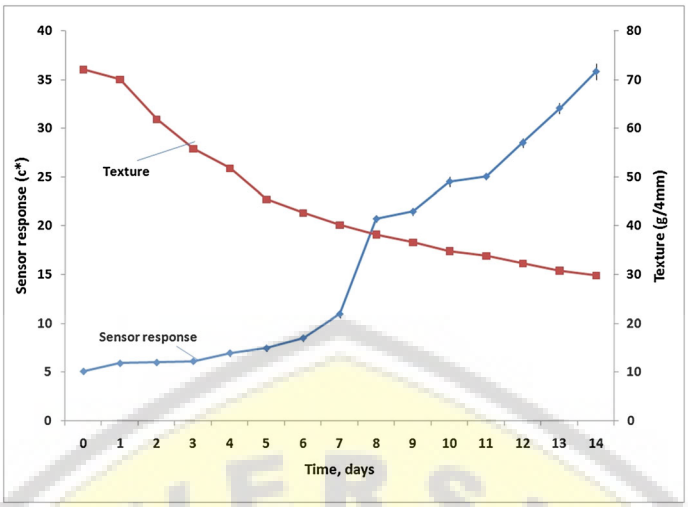

Figure 9. The texture values of chicken meat samples and the sensor response at chiller temperature ($n = 4$).

Preparation of bacterial cellulose membrane

Acetobacter xylinum culture (coming from the Pure Culture Collection of the Chemistry Department, University of Jember) was cultivated in stationary conditions using a Herstin–Schramm nutrient (HS) medium composed of glucose (2 w/v%), yeast extract (0.5 w/v%), bacto-pepton (0.5 w/v%), citric acid (0.115 w/v%), Na_2HPO_4 (0.27 w/v%), $MgSO_4 \cdot 7H_2O$ (0.05 w/v%) and ethanol (1 v%) added after sterilization of the base.[27,28]

Conic flasks (300 cm^3) were used, filled with an HS medium. The bacterial breeding process was conducted within 7 days at 30°C, grafting inoculums of approximately 4 w% in relation to the medium prepared. In the process of bacterial cellulose biosynthesis, glucose as well as arabinose, mannose, galactose, xylose and mannitol was used as carbon sources. The membrane of the bacterial cellulose obtained was then treated with NaOH (a concentration of approximately 5%, for 60 min, temperature = 100°C) in order to remove bacterial cells and substrate from the inner layers of the

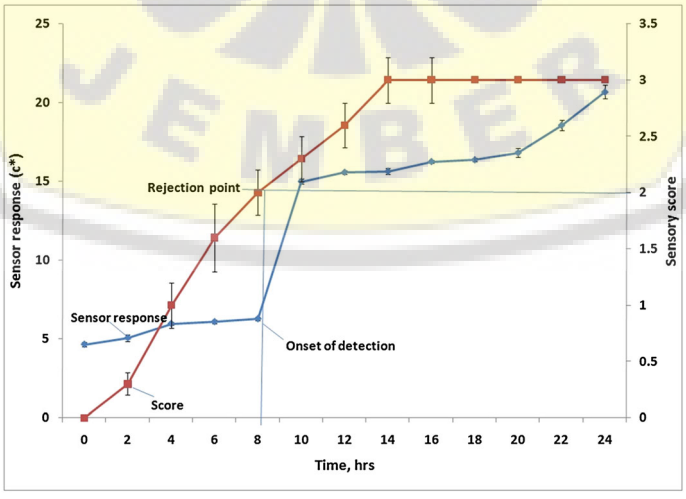

Figure 10. The sensory score of chicken meat samples and the sensor response at room temperature ($n = 4$).

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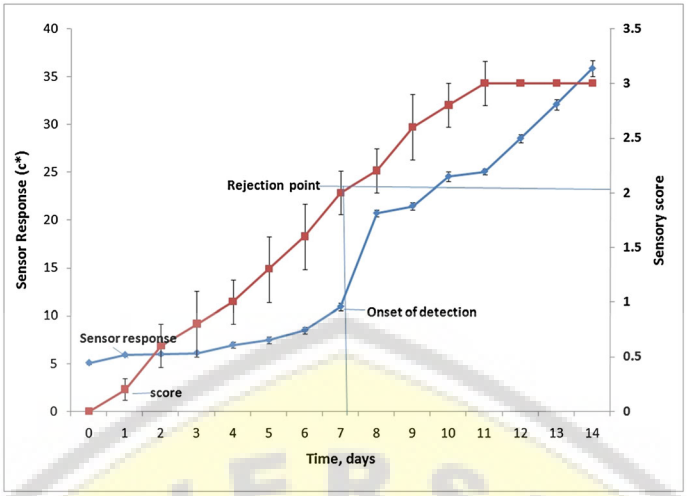

Figure 11. The sensory score of chicken meat samples and the sensor response at chiller temperature ($n = 4$).

Table 1. The results of sensory evaluation of chicken meat samples at room temperature.

Stored time (h)	Colour	Odour	Colour of sticker sensor
0	Fresh pink	No peculiar smell	Red
2	Fresh pink	No peculiar smell	Red
4	Pink	No peculiar smell	Red
6	Pink	No peculiar smell	Red
8	Red	Light peculiar smell	Orange
10	Red	Smelly	Pale orange
12	Deep red	Slightly foul	Yellow
14	Deep red	Foul	Yellow
16	Deep red	Foul	Yellow
18	Deep red	Foul	Yellow
20	Deep red	Foul	Yellow
22	Deep red	Foul	Yellow
24	Deep red	Foul	Yellow

Table 2. The results of sensory evaluation of chicken meat at chiller temperature.

Stored time (day)	Colour	Odour	Colour of sticker sensor
0	Fresh pink	No peculiar smell	Red
1	Fresh pink	No peculiar smell	Red
2	Fresh pink	No peculiar smell	Red
3	Fresh pink	No peculiar smell	Red
4	Pink	No peculiar smell	Red
5	Pink	No peculiar smell	Red
6	Pink	No peculiar smell	Red
7	Red	Light peculiar smell	Orange
8	Red	Light peculiar smell	Pale orange
9	Red	Smelly	Yellow
10	Deep red	Slightly foul	Yellow
11	Deep red	Foul	Yellow
12	Deep red	Foul	Yellow
13	Deep red	Foul	Yellow
14	Deep red	Foul	Yellow

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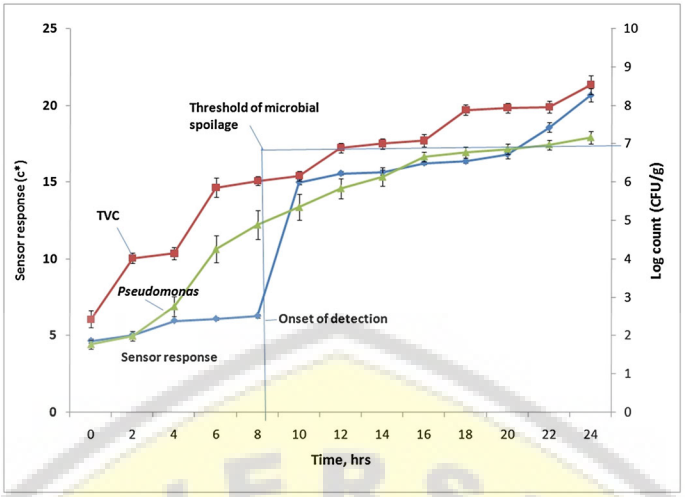

Figure 12. The TVC and *Pseudomonas* count of chicken meat samples and the sensor response at room temperature ($n = 4$).

bacterial cellulose film. Then, it was rinsed with tap water until a neutral condition (around pH 7.0) was achieved.

For membrane sheet preparation, the bacterial cellulose (10 g) was blended until homogeneous and then cast onto the glass plate and pressed. Afterwards, the membrane was left overnight (12 h), and the membrane sheet was dried at 60°C. The membrane sheet was stored for further use.

Immobilization of methyl red on bacterial cellulose membrane

The methyl red was immobilized on bacterial cellulose membrane by absorption method. This procedure was carried out simply by immersing the membrane sheet into 10 ml of a stock solution of methyl red (1 mg/ml) overnight (12 h) at ambient temperature. Then, the methyl red/cellulose was washed with tap water to remove any unbound indicator within the membrane. The methyl red/cellulose membrane was dried using an electrical drier. Afterwards, the methyl red/cellulose membrane was cut into the desired shape according to the design as an on-package sticker sensor (Figure 1).

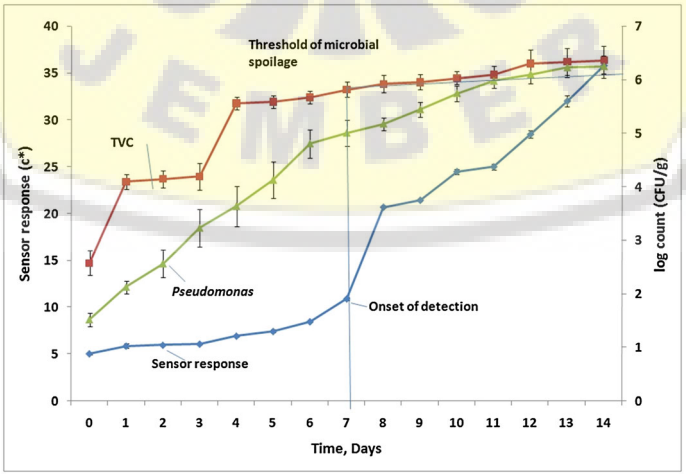

Figure 13. The TVC and *Pseudomonas* count of chicken meat samples and the sensor response at chiller temperature ($n = 4$).

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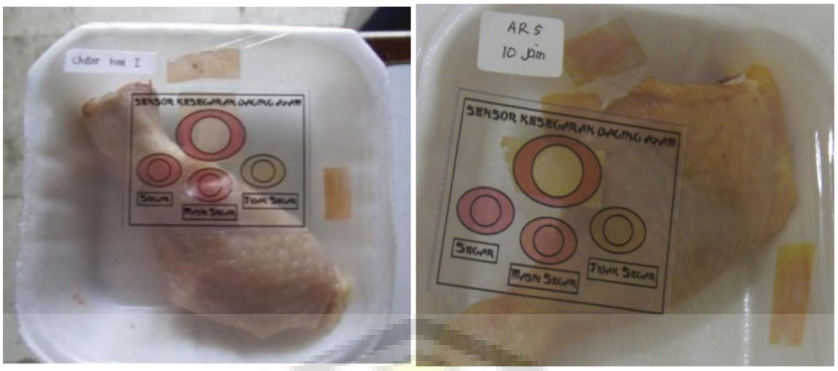

Figure 14. Application of methyl red/cellulose membrane as a visual sticker sensor for chicken meat freshness (left: at 2 h, and right: after 10 h at room temperature).

Preparation of broiler chicken cut samples

A fresh broiler chicken of normal pH (5.5–5.6) purchased at a local poultry meat shop (at Jember) was used in this study. The broiler chicken was cut into portions of 100 g and 50 g for microbiological and sensory analyses respectively. Then, the portions were placed on plastic trays and enclosed with a low-density polyethylene plastic film (0.9 g/cm^3; Carrefour, Indonesia). The samples were stored under chiller conditions (4 ± 0.2°C) in a low-temperature incubator (model MIR 153; Sanyo Electric Co., Japan) and in room temperature (28 ± 2°C). The temperature of the samples was monitored throughout the entire storage period by using electronic temperature recording devices (Cox Tracer, Belmont, NC). Four sample packages of the chicken cut product were taken at appropriate time samples for each storage temperature. All four samples were analysed for microbial growth, pH and sensory characteristics of colour and odour; for increased accuracy, each sample determination was replicated three times (12 determinations in total per test condition). For purposes of statistical analysis, the average value of the three determinations was used per sample such that the statistics describe the variation between samples with $n = 4$.

Microbiological analysis

Samples (25 g) of chicken cuts were aseptically weighed, added to quarter 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 quarter 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 (a) total aerobic viable count (TVC) on plate count agar (PCA; Merck), incubated at 25°C for 72 h, and (b) *Pseudomonas* spp. On cetrimide–fucidin–cephaloridine (CFC) agar (Oxoid, CM559 supplemented with selective supplement SR 103E, Basingstoke, UK) incubated at 25°C for 48 h. 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.

Measurement of pH and VA in broiler chicken cut samples

The pH values were recorded by a pH meter (Russel, Model RL150), with the glass electrode being immersed in the homogenate of chicken meat after the end of microbiological analysis. Perchloric acid extracts of the chicken meat samples were prepared and analysed for TVBN levels according to Pearson.[29] All the chicken meat samples were washed thoroughly with tap water. The chicken meat from one side was skinned aseptically and minced by passing it three times through a meat grinder with 4 mm holes. 10 g of chicken meat sample was blended with 90 ml of PCA 6%. 50 ml of filtrate was

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made alkaline with hydroxide 20% and distilled for 10 min in a 2100 Kjeltec Distillation Unit (FOSS Tecator AB, UK). Each analysis was repeated three times.

Sensory analysis

Sensory evaluation of chicken cut samples was performed during storage under chiller or room conditions, by a five-member sensory panel composed of staff from the laboratory. The same trained persons were used in 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 temperature. The meat product was evaluated. Special attention was given to the colour, texture and odour of the chicken meat. The texture of the chicken meat was measured using a texture meter (Rheotex, UK). Odour was judged and recorded in appropriate forms with descriptive terms, reflecting the organoleptic evolution of quality deterioration.[30] A simple three-point scoring system was adapted.[30,31] Each attribute was scored on a continuous 0-to-3 hedonic scale, with 0 being the highest quality score, 1 given to the acceptable product, 2 being the limit of product acceptance or rejection point and 3 the unacceptable chicken cut sample.

Measurement of the visual sticker sensor response

The visual sticker sensor consisted of methyl red immobilized on bacterial cellulose and designed as given in Figure 1. The sticker sensor was placed on the packaging of the chicken cut samples, in direct contact with the atmosphere in the package via a hole that attached to the sensor. The chicken cut packages were stored at chiller and room temperature in order to evaluate the applicability of the developed sticker sensor to monitor the spoilage process of chicken cuts.

The distinct irreversible colour change of the sensor from the initial red to yellow was used as the measurable response of change. The kinetics of colour change of the sticker sensor was assessed using a hand-held colorimeter (chroma meter CR-10, Minolta Inc., Japan) to determine the CIE colour space co-ordinates, i.e. the visible colours 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 colour intensity) were calculated as $[(a^{*2} + b^{*2})^{0.5}]$. Here, for simple measurement, c* (colour intensity) is used as sensor response for the intensity of yellow colour of sticker sensor (in arbitrary units) in all experiments.

RESULTS AND DISCUSSION

Response of sensor towards broiler chicken cut spoilage

All sensors were placed proximately (3 cm) from the chicken cut samples in order to respond to the increase of VAs generated by spoilage with a very distinct colour change from red to yellow. The sensors were monitored periodically until no further colour change was observed. Figures 2 and 3 show the rate of colour change of the sensor (c*, intensity of yellow in arbitrary unit) towards spoiling meat at room and chiller temperatures. In Figure 2, the sensor response increased steadily (as the sensor colour changed to yellow) within 24 h of the experiment, observed at room temperature. Here, the sensor gradually changed colour from red to yellow after 8 h at room temperature and increased afterwards (as the yellow colour appeared) until the 24th hour of experiment. While at chiller temperature as shown in Figure 3, prior to the first 3 days, no drastic colour change was observed. Then, after day 7, the sensor changed to yellow. In general, the sensor changed colour from red to orange after 3 days, and then changed to yellow after day 7 at chiller temperature. Furthermore, visual inspection did not detect any differences in colour changes between the sensors of the different sample batches as also evidenced by no difference in sensor colour (c*). The onset of spoilage was detected after 8 h and after 7 days for room and chiller temperatures respectively. This indicated that the chicken

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cut samples released VAs at a relatively slow rate, since its freshness lasted longer within 10 h and 8 days for room and chiller temperatures respectively.

pH and TVBN analyses

Figures 4 and 5 show the pH values of the chicken cut samples along with the sensor response. The pH values of the chicken cut samples varied from pH 5.60 at the fresh stage to pH 6.18 at the spoilage stage at 10 h at room temperature (Figure 4). The pH values of the chicken cut samples varied from pH 5.61 at early day to pH 6.16 at day 7 at chiller temperature. It can be seen from both figures (Figures 4 and 5) that the sensor also follow a similar trend as shown by the pH responses under both conditions. Furthermore, the sensor also responded to the increase in pH value in the package headspace, since the range of methyl red/cellulose membrane colour change was related to the pH levels of the chicken cut samples.

According to Dainty,[12] meat spoilage occurs at high pH (>6.0), at lower cell densities, than at normal pH (<5.8) for fresh meat. This value has been reached after 8 h and after 7 days of storage at room and chiller temperatures respectively. Thus, the sensor gave an accurate response that the indication of spoilage was also detected after 8 h and after 7 days for room and chiller temperatures respectively.

TVBN as VA levels rose due to the formation of NH_3 and other VAs. BAs such as histamine, putrescine, tyramine and cadaverine have been implicated as amine indicators of meat product decomposition.[16,18] The concentration of produced ammonia was found to be proportional to the concentration of BAs and could hence be used to determine BAs in food matrixes.[32] Byun *et al.*[21] have been proposing TVBN as indicators of meat quality, with TVBN limit values of approximately 20 and 30 mg N per 100 g for beef and pork respectively.

The results for this TVBN measurement are given in Figures 6 and 7 along with sensor responses at room and chiller temperatures 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 colour change was related to the levels of TVBN in the chicken cut samples. The freshness decreased with increases in TVBN. The TVBN value for hygienic standard for fresh (frozen) livestock meat is ≤20 mg/100 g,[33] whereas for chicken, TVBN values are higher. Therefore, these levels were reached after 8 h and after 7 days at room and chiller conditions respectively, which is similar with the sensor responses given in Figures 6 and 7, where the sensor indicated that the packaged chicken cuts were spoiled or have deteriorated after 8 h and 7 days at room and chiller temperatures respectively.

Sensory analysis

The colour, texture and odour of chicken cut samples were first evaluated by sensory evaluation, and the results were recorded. The measurements were conducted along with the sensor response, and the results were also recorded. The results of the sensor response were therefore confirmed by the sensory evaluation. The measurement was done under laboratory conditions, without any special requirements considering application in shopping centres, restaurants, storage rooms and other places. Figures 8 and 9 show the average values of the texture readings from the Rheotex meter used for the measurements of chicken meat samples at room and chiller temperatures respectively. Each piece of data is the average of three measurements under identical conditions. It can be seen from both figures (Figures 8 and 9) that the sensor response showed a similar trend with texture value. The freshness decreased along with decreases in the texture of the chicken cut samples and the change in the sensor colour from red to yellow.

Figures 10 and 11 show the output score of the odour measurement corresponding to Tables 1 and 2. Tables 1 and 2 list the results of the sensory evaluation in room and chiller temperatures respectively. From both figures, it can be seen that the sensor response is similar 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 was indicated by the colour change of the methyl red/cellulose membrane to yellow, indicating spoilage, as given in Tables 1 and 2 for room and chiller temperatures respectively.

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Microbial analysis

The TVC counts steadily increased from 8.4×10^3 cfu/g during the initial 2 h to 6.9×10^5 cfu/g at 10 h of investigation at room temperature (Figure 12). Initially, the *Pseudomonas* counts were at approximately 70% of the TVC counts, rising to approximately 80% at 24 h at room temperature. Here, the *Pseudomonas* counts increased sharply until the 16th hour of investigation, with approximately 3.0×10^8 cfu/g at room temperature. At chiller temperature, initially, the TVC counts were at 5.7×10^3 cfu/g at day 1, rising to 6.7×10^5 cfu/g at day 8 at chiller temperature (Figure 13). The *Pseudomonas* counts were at approximately 60% of the TVC counts, rising to approximately 80% at day 7 under chiller conditions. Then, they increased steadily and similarly to the TVC count under chiller conditions. When compared to the sensor response in both figures, it can clearly be seen that not only did the sensor response correlate with the changes in bacterial populations but also that the sensor colour change from red to yellow correlated 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 product.[34] These levels were reached at 10 h and 8 days under ambient and chiller conditions respectively. Thus, the sensor accurately responded to the increase in volatile base concentration in the package headspace both at room and chiller temperatures. In general, the range of the sensor colour change could be related to the higher levels of microbial population in the chicken cut samples. In addition, the visual colour changes of on-package sensors are useful indicators of the approximate microbial population and, therefore, the spoilage of the chicken cut samples (Figure 14). Finally, it can be clearly stated that this sensor can be used to indicate the presence of high microbial populations in packaged chicken meat, when the colour of the sensor changed from red to yellow during visual identification, suggesting that the chicken meat has spoiled and cannot be consumed anymore.

CONCLUSIONS

A methyl red/cellulose membrane was used to develop an on-package sticker sensor, and the relationship between the sensor and the chicken cuts' freshness quality was investigated. The test results showed that the sensor could be used for assessing chicken cuts' freshness, as the relationship between the colour change of methyl red/cellulose membrane, as a sensor response, and the storage time of chicken cuts follows a similar trend, and the spoilage of the chicken cuts could be detected clearly (when the sensor changed to yellow). A sticker sensor responds properly to chicken cuts' freshness as shown by its colour change towards the deterioration of chicken cuts in real time. Thus, the sticker sensor can be used as an effective tool for the real-time monitoring of the microbial quality of packaged fresh meat. The sticker sensor may serve as active shelf-life labelling devices in conjunction with the "used-by-date" labelling, when attached to individual product units, or may be used to optimize distribution control and management of the stock rotation system and in reducing food waste.

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