Colorimetric Paper-Based Dual Indicator Label for Real-Time Monitoring of Fish Freshness

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SUMMARY

Research background. Fish freshness and quality monitoring are of high importance for consumers, retailers, and companies. Therefore, developing novel approaches that are simple, fast, non-destructive, and inexpensive to monitor fish freshness in real-time is of great value. One alternative is using Intelligent or smart packaging to monitor the freshness level or condition of packaged fish.

Experimental approach. On-package dual indicator label based on paper-based pH sensors was developed for real-time monitoring of the milkfish (Chanos chanos) freshness. The paper-based pH sensor was prepared using bromocresol purple (BCP) and bromothymol blue (BTB) that were immobilized onto filter paper via dip-coating. Herein, the fish degradation could be monitored visually by the dual indicator label, where the BCP change from yellow to pink, then finally to purple, while the BTP change from orange to green-yellow, and finally to green-blue for fresh, medium, and spoilage respectively.

Results and conclusion. The label responds sensitively to the fish freshness, in terms of its pH change due to the fish degradation as presented by the dual indicator color change to indicate the fish freshness status at room and chiller conditions. This pH change was followed by changes in the other parameters related to freshness, such as TVBN (total volatile

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basic amine), TVC (total viable count), texture, and odor evaluation. The threshold of fish spoilage indication was observed at 8 h in room temperature and 7 days in chiller condition as the deterioration time point was met with color changes. Thus, it can be concluded that the dual indicator label can be applied as a simple and low-cost on-package active label for fish freshness monitoring.

Novelty and scientific contribution. Increasing consumer concerns for quality and safe food worldwide has boosted the search for a novel approach to food monitoring. In this work, a simple and practical on-package dual indicators label for real-time monitoring of fish freshness was developed. The colorimetric pH sensor was fabricated simply via dip-coating of filter paper, and yet easy detection using a naked eye for accurate fish spoilage. The dual indicators label changes color in a similar tendency toward other freshness parameters, such as TVBN, TVC, texture, and odor.

Keywords: dual indicators; pH dyes; colorimetric sensor; fish freshness; intelligent packaging

INTRODUCTION

Fish is a high-value and healthy food that is consumed daily by many people due to its high nutrient value and good taste (1). However, storage conditions and treatment after catching the fish can affect strongly its freshness and quality (2,3). Hence, fish freshness and quality monitoring are of high importance for consumers, retailers, and companies. Commonly, the classical methods for the examination of fish freshness are chemical (4), microbial techniques (5), or sensory evaluation (6), which need long analysis time, laborious procedure, and skilled operators (7,8). New and rapid techniques, such as electronic noses (9,10) and hyperspectral imaging (11,12) have also been proposed currently. However, these techniques need complicated instruments and are expensive. Thus, developing novel approaches that are simple, fast, non-destructive, and inexpensive to monitor fish freshness in real-time is of great value.

Intelligent or smart packaging is a small, low-cost label attached to food packaging to monitor the freshness level or condition of packaged food (13,14). Commonly, intelligent packaging has an indicator attached to the package that allows color changing via reacting with the chemical compounds released from the packaged food (15). For instance, the pH dye (bromocresol green) has been developed as an intelligent label by entrapped within a polymeric membrane to detect fish freshness (16). Moreover, other studies based on pH change detection have also been reported recently (17,18,19). Herein, a single indicator or sensor was used as an active label for food freshness monitoring. For a more accurate label
for food freshness detection, other approaches were proposed by using mixed pH dyes as an active label for spoilage detection of skinless chicken (20) and a dessert (21). Even though this label consists of two or three mixture pH dyes (20), However, these labels were still employed as a single sensor or indicator. The drawbacks with a single indicator are like traditional acid-base titration with an indicator dye. It is often hard to define when the end of titration is reached, sometimes, it is too early or too late. Similarly, with single freshness indicator, the onset of detection associated with the threshold of spoilage not easy to determine, caused too early or too late in the detection of spoilage threshold, and other freshness levels (22).

To avoid these disadvantages of a single indicator used, the dual indicators based on dual pH dyes are developed as a novel approach for the dual sensors label for beef freshness monitoring (23). Based on this approach, the dual indicators label for on-package fish freshness monitoring is developed. The label has been fabricated based on paper-based pH sensors using bromocresol purple (BCP) and bromothymol blue (BTB). Here, the paper-based pH sensors contain suitable pH-sensitive dyes; the selected synthetic dyes have several advantages over natural indicators due to the ease and convenience of synthesis, strong color response, environmental stability, pH sensitivity, and a lack of odor. Furthermore, in comparison with using a single pH dye, a combination of pH indicators (BCP and BTB) can effectively improve the pH sensitivity to prevent indistinguishable transition colors during the color change. While, paper-based sensors can be fabricated simply by casting, coating, or inkjet printing specific reagents, such as pH indicators or complexing agents, onto cellulose-based filter papers (24, 25). They are flexible, sensitive, low-cost, and easy to make the desired design of freshness label as well as provide the advantage of point-of-need sampling for application in the field, such as in this intelligent packaging, as fish freshness monitoring.

In this work, the goal of this work is to construct two pH dyes (BCP dan BTB) as an on-package dual indicators label for real-time monitoring of fish freshness. Since both pH dyes are well known as sensitive indicators in the pH range >7.0, where fish deterioration occurs (26). Herein, the dual indicators label response toward fish freshness levels at different temperatures (room and chiller) was correlated with other fish freshness properties, such as pH, TVBN, TVC, texture, and odor evaluation, to show the reliability of the label in the real-time fish freshness monitoring.

MATERIALS AND METHODS

Chemicals

The dyes stock solution of bromocresol purple (BCP) and bromothymol blue (BTB) (Sigma, Dorset, UK) was prepared by dissolving 10 mg of BCP and BTP in 10 mL of ethanol.
(50 %) to make 1 mg/mL concentration of dyes respectively. All chemicals used were of analytical grade and used as purchased without further purification.

**Preparation of dual indicators label**

The BCP was immobilized onto filter paper (Whatman, No. 1001-325, Merck, Massachusetts, USA) via absorption using a dip-coating technique to create a BCP membrane via the adsorption method. The dip-coating was performed by dipping or immersing the paper (in the desired shape), into 10 mL of BCP stock solution overnight (12 h) at room condition. Afterward, the BCP membrane was washed with deionized water to remove the unbound dye within the membrane. Then, the BCP membrane was completely dried with aid of an electrical drier. The same dip-coating procedure was also applied for the BTB immobilization onto paper as the BCP membrane. Afterward, the BCP and BTB membranes as colorimetric paper-based pH sensors were placed on the fish package, as on-package dual indicators label according to the desired fish design label (Fig. S1).

**Preparation of fish samples**

Fresh milkfish (*Chanos Chanos*) with a pH = 6.20 to 6.25 was supplied from a local fishpond (Puger, Jember) at a similar size and mass (*l*=28 cm, *m*=195 g) and packaged using an iced styrofoam box for fish and send to the laboratory within 25 min. The fish is then cut into two pieces so that the fish can be placed easily in a styrofoam tray (*I*=20.5 cm, *m*=19 cm, *h*=5 cm). Herein, similar parts and conditions of fish were used in terms of their freshness, mass, and size. Then, the trays were covered with PE film commonly used for food packaging. Afterward, they were stored in the incubator (model MIR 153, Sanyo Electric Co., Osaka, Japan) in a room (28±2 °C and chiller (4±0.2 °C temperatures. Then, the temperature was monitored via electronic temperature devices (Cox Tracer; Belmont, NC, USA) during the storage period. At both temperatures, triplicate fish samples were tested at a selected interval time for kinetic analysis of the fish freshness level, *i.e.* chemical and microbiology assays, and odor evaluation for the fish spoilage study stored under both conditions. For microbiological analysis and odor evaluation, the fish was cut into two portions (~97 g). All measurements were performed in triplicate.

**Characterization of the dual indicators label**

The two indicator labels were then compared to a plain filter paper used as a substrate, and the BCP and BTB membranes in terms of moisture absorption, and Scanning Electron Microscopy (SEM) morphology. The detection of moisture absorption (%) was conducted
according to the previous method (27) with a slight adjustment. The BCP, BTB membranes, and the paper substrate were cut by a hole punch, into a round shape (d.a. 2 cm). Then, they were placed inside the chamber (Conway diffusion cell), sealed with a cap, then stored under 30 °C. Afterward, the outer chamber was filled with saturated NaCl solution (10 mL). A relative humidity (RH) of 76 % was maintained inside the cell. The membrane masses were determined every 12 h over a while till constant, then the moisture absorption ($w$) was calculated as follows:

$$w(\%) = \left[\frac{(m_t - m_i)}{m_i}\right] \times 100\%$$

where $m_t$ is the membrane mass (mg) at a certain time interval, and $m_i$ is the initial membrane mass (mg). Three membrane samples were measured and the moisture adsorption (%) was calculated as an average (the standard deviation).

SEM images of the membrane surface morphologies were determined at a voltage of 3kV using an S-4800 SEM (Hitachi, Tokyo, Japan). Before the SEM image of each membrane sample was obtained, the samples were prepared by cutting the membrane into small pieces and sputtering with gold to make the samples conductive. Three membrane samples including plain filter paper, BCP, and BTB membranes were examined.

The dual indicators label response

The dual indicators label was placed inside the package of the fish samples, where the label was placed in contact with the package headspace, while the reference label for reading the freshness degree was placed outside the package, just above the dual indicators. Afterward, they were stored in room and chiller conditions, to evaluate the dual indicators label performance for fish spoilage monitoring.

Since the dual indicators label could be detected by the naked eye; the color changes of the label were taken by scanometric method (28, 29) using a flatbed scanner (Canoscan, LIDE 110, Tokyo, Japan), where the color image resolution used for scanning was 300 dpi. Then the ImageJ® program for Windows® (29) was employed for analyzed the color value. The label color response development was presented as a mean RGB value. Herein, all of the measurements were measured in triplicate.

pH, volatile amine, and microbiological analysis

The fish sample pH values were measured by a pH meter (Model RL150, Russell, UK), where the glass electrode was immersed in the homogenate fish sample solution measured in triplicate. The TVBN values were prepared and analyzed using perchloric acid (PCA) extract from the fish samples according to Person (30). All the fish samples were thoroughly washed with tap water. Then, the fish were aseptically skinned and minced by passing three times via
a grinder (4 mm holes). The fish samples (10 g) were blended with 90 mL of PCA (6 %) and then the filtrate (50 mL) was alkalinized with hydroxide (20) and distilled in a Kjeltec™ 2100 Distillation Unit (FOSS Analytical, Sangerupgate, Denmark) for 10 min (31). The TVBN analysis was performed in duplicate.

The microbiological analysis was measured according to Kuswandi et al. (31) with slight modification. The fish sample (25 g) were weighed aseptically, then added to strength Ringer's solution (225 mL), then homogenized in a stomacher (Lab Blender 400, Seward Medical, London, UK) for 1 h at room temperature. Serial dilutions in strength Ringer's solution were made and duplicate samples (1 mL) of proper dilutions were spread on the surface of the media in Petri dishes for enumeration of total aerobic viable count (TVC) using Plate Count Agar (PCA; Merck, Darmstadt, Germany), and incubated at 25 °C for 72 h. The plate was visually inspected for the typical colony and morphological types that were associated with the growth medium. Colonies were calculated and presented as log CFU/g.

Odor and texture

The fish sample sensory evaluation was examined at chiller or room temperatures and tested using 20-member trained panels consisting of students from the department. The panelists were trained to select their preferences objectively according to the odor acceptance of the fresh fish samples. Since odor was evaluated in proper forms that presented the organoleptic change of quality degradation (32). The odor training session was given using different fish statuses (fresh, medium, and spoilage), where 5 fish samples were used for each status. The trained panels were employed in each fish odor evaluation, blinded to the fish sample history regarding time and temperature. The odor evaluation was performed in similar conditions, e.g. ventilation and lighting, where the light and the temperature of packaged fish samples are like the ambient conditions. The fish sample freshness was determined especially to the odor in terms of like (1) and dislike (0) (33). So if all panels judge is like the fish odor or odor acceptance (%), meaning it is fresh (100%). On the contrary, if all panels' judge is disliked or rejected, meaning it is spoilage (0%) the medium status of odor acceptance will be more than 50% or more of the panelist member. In the case of fish texture softness, the measurement was performed using a texture meter (TA-XT2i, Stable Micro Systems, Surrey, UK) for a textural profile test consisting of fish softness, which was used to compress fish meat between two parallel plates at a crosshead speed of 1 mm/s to give a more objective evaluation.

Statistical analysis
The statistical analysis of data (means±standard deviation) of Moisture absorption properties of plain filter paper (control), and immobilized paper with BCP and BTB were processed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). An analysis of variance (One-Way ANOVA) using the linear model procedure was performed. Descriptive and Tukey’s multiple range tests were conducted for comparing the means at a 5 % significance level.

RESULTS AND DISCUSSION

Characterization of the dual indicators label

Filter paper is a common substrate for fabricating indicator labels because its readily available and low cost. Herein, the sensing membrane was fabricated by the dip-coating technique of filter paper with pH dye via simple adsorption as described above. The fish design label was employed, since it is simple in preparation and construction, including easy to distinguish by the naked eye for freshness level. The simple preparation due to the filter paper used as a substrate for pH indicator immobilization can be easily cut using a scissor or cutter for the desired shape, like fish in this case. Both parts (head and tail) can be immobilized with the BCP and BTB respectively. Finally, both pH membranes in the dry condition could be constructed to produce a paper-based pH sensor with the aid of transparent double tapes, where one side for forming the dual indicator label, and the other side to attach inside fish packaging. Herein, for simple colorimetric detection, the label can be detected easily by the naked eye for each fish's freshness level (i.e. fresh, medium, and spoilage). Furthermore, this label construction prevents the two indicators used to diffuse each other inside the filter paper, which in turn, could change the label color. As both BCP and BTB membranes were dry completely, both membranes were separated using separate filter paper, and transparent double tapes were used only to hold the membranes in their position.

Moisture absorption is one of the important characteristics of a colorimetric indicator. When a colorimetric indicator is attached to the packaging headspace, the relative humidity (RH) at a high level in the package might gave a negative effect on the indicator color change, which can lead to an inappropriate response. Since, after moisture absorption, the colorimetric indicator might be swelling or damaged. Therefore, moisture absorption by the indicator is allowed at a low level. Herein, the moisture absorption of all three samples increased and reached saturation over time. The moisture absorption at 60 h (at 76 %RH) for the filter paper, the BCP, and BTB membranes were 15.2 %, 9.2 %, and 9.5 %, respectively (Fig. 1a). It was found that the moisture absorption of the indicator membranes (BCP and BTB) was almost 2 times lower than the filter paper (p < 0.05). This finding of the moisture content of the indicator membrane (> 100 %) is enough to reduce moisture from interfering with the...
indicator's response in terms of its color change (35,36) when the indicator membranes are employed as a label for the real-time fish freshness monitoring.

The SEM images of three samples (filter paper, BCP, and BTB membranes) under magnifications of 50K times were given in Fig. 1b. It can be seen that the three surfaces of the sample tested (Fig. 1b (i), (ii), and (iii)) were differences between the filter paper as substrate (i), and the surface structures of immobilized papers with BCP (ii) and BCP (iii) membranes as pH indicator labels. Therefore, it can be stated that the paper was evenly bound on the surface of filter paper so that it could bind BCP and BTB dye molecules tightly with good stability (37). Moreover in the BCP and BTB membranes, fibrous and fluffy surfaces are also appeared, so that they are serving a larger surface area to interact instantaneously with the released gas from the fish sample (38). Thus, the surface properties of both indicator membranes are suitable for an active label application.

Response of dual indicators label and pH

All dual indicators labels were attached inside the headspace packaging close proximate to the fish samples so that it allows for interaction with the volatile amine produced by degraded fish samples with a very distinct color development of the BCP membrane (head indicator) from yellow to pink, then finally to purple, while the BTP membrane (tail) develop color from orange to green-yellow, and finally to green-blue for fresh, medium, and spoilage respectively (23,39). The label was inspected periodically till no further color development was detected. The rate of color changes of the dual indicators label response toward fish spoilage at room and chiller temperatures respectively are presented as mean RGB values (Fig. 3a and Fig. 3b). Herein, both the BCP and BTB membrane response declined sharply up to 8 h, then afterward they change slightly up to 24 h of the experiment, where BCP was almost steady after it changes to purple up to 24 h at room temperature as depicted in Fig. 2a. In the chiller temperature, both BCP and BTB membrane developed color sharply at the first 4 days, then gradually up to day 7, and gradually developed up to 14 days, where BCP was almost stable after it developed to purple (Fig. 3b). Moreover, the visual detection by the naked eye did not find any variation in color development between these labels of different batch preparation in both conditions. Thus, it can be stated that as far as the fish samples have the same freshness status that roughly produces the same number of volatile amines, which in turn, caused roughly the same pH change inside the headspace of the fish package, thus the dual indicator label color development will also the same. The onset of fish spoilage was found at 8 h and 7 days at room and chiller temperatures respectively (38,40). The results also show that volatile amines were produced gradually from the fish samples during the deterioration process.
Herein, by developing the dual indicators label as a real-time active label, the false-negative and false-positive could be prevented, as both indicator membranes were referenced each other, prevented false or error of each indicator response, and placed inside the headspace of the fish package, so that only direct contact with the headspace of the package for fish freshness monitoring, and no effect from outside of the ambient environment (23). The false negative might happen if the plastic cover was opened or broken. Since the volatiles amines released during fish sample degradation would leave out, caused its concentration inside the headspace would reduce significantly, making the different response of the label. Hence, keeping the package in the right place, and the label in its position is compulsory for reliable monitoring of fish freshness (23).

The precision of the dual indicators label response toward fish freshness is shown with the reproducibility of the color development as the error bars (Fig. 3a and Fig. 3b), where their values were below 2 % which is excellent for this measurement (41). Furthermore, the robustness of the label was evaluated in the different batch preparation and days to evaluate their response toward fish freshness (41). The results found that their responses were consistent with different fish freshness statuses.

The pH values of the packaged fish samples along with the dual indicator label responses at room and chiller temperature respectively are presented in Fig. 3c and Fig. 3d. Herein, the pH values of the fish sample were increased steadily from pH 6.20 at the fresh status to pH 7.53 for the threshold of spoilage at 8 h, while the dual indicators label also develop their color as the onset of detection at room temperature (Fig. 3c). Whereas at chiller temperature, the pH values of the fish sample were increased steadily from pH 6.25 on day 1 to pH 7.56 on day 7 as a pH of fish spoilage threshold, as well as the onset of detection for the dual indicators label to develop their color (Fig. 3d) (42, 43). According to Fig. 3, both indicator membranes also show decreased color value (mean RGB) as pH increased at both temperatures. This is due to the range of both BCP and BTB indicators being similar to the pH change in the fish sample freshness status. Generally, spoilage of fish occurs at high pH (> 7.0), then at normal pH (< 6.0) of fresh fish (2, 4). Herein, this pH value has been achieved at 8 h and 7 days at room and chiller respectively (40, 38). Hence, the labels show a correct response on the onset of spoilage (fish spoilage just started), where it was detected at 8 h and 7 days at room and chiller respectively.

**TVBN and microbial analysis**

Commonly in spoiling fish, the volatile basic amine (TVBN) value increased because of the NH₃ production, including other volatile amines, as a result of protein degradation.
Moreover, fish deterioration can be detected by the production of biogenic amines (e.g., histamine, tryptamine, tyramine, spermidine, spermine, cadaverine, and putrescine), produced post-mortem in fish and shellfish products (44). These biogenic amines are low-molecular-mass aliphatic, alicyclic, or heterocyclic organic bases that originated from the specific free amino acids decarboxylation in fish or shellfish tissue (45,3). The use of TVBN as objective product standards or the indices of fish quality has long been used and suggested, due to the tests being rapid if compared to microbiological analyses and less subject to individual interpretation than sensory analyses (46).

Fig. 4a and Fig. 4b show that the TVBN value increased from 7.355 for a fresh condition to 32.376 (mg/100g) as a threshold of spoilage at 8 h at room temperature, and 7.311 to 30.288 (mg/100g) at 7 days at chiller temperature, as the on-set of detection for the dual indicators start to develop color. It can be stated that reducing the color of the dual indicator label is in a similar trend toward increased TVBN during the monitoring period. Hence, the dual indicators properly respond to the TVBN increase in the fish package headspace, as the range of dual indicators’ color development is associated with the TVBN value in the fish samples. Herein, the fish freshness decreases when TVBN increases, where a TVBN value for fresh fish is a limit of < 35 mgN/100g fish for many various species (47). The TVBN values were detected at 8 h and 7 days as the onset of detection, where the dual indicators label showed a color change that the packaged fish was spoilage under both conditions (Figs. 4a and Fig. 4b) (48). Thus, it can be stated that the dual indicators label can be employed as a simple and effective active label to indicate the high TVBN value in packaged fish, by their color change, where it can be detected using the naked eye as an indication for fish spoilage.

The microbial analysis of the fish sample was presented as TVC counts (Fig. 4c and Fig. 4d). According to this figure, during the first 2 h, the TVC steadily increased from 4.213 log CFU/g to 5.487 log CFU/g at 8 h; as a threshold of fish spoilage at room temperature, where this point is also an onset of detection for the dual indicators label to develop color distinctively (Fig. 4c). While at the chiller, the TVC counts show initially at 3.960 log CFU/g at day 1 and increased to 5.875 log CFU/g on day 7 as a threshold of fish spoilage, and as the onset of detection for the dual indicators label to develop their color distinctively (Fig. 4d). Based on Fig. 4c and Fig. 4d, it is shown that the reduced dual indicators color response is in a similar trend to the increase in bacterial counts. The threshold limit value of TVC ≤ 7.0 (log CFU/g) was used for bacterial spoilage of fish in many studies (12). Herein, the TVC value used for the fish rejection is slightly earlier and achieved at 8 h and 7 days at room and chiller conditions respectively. Hence, this value prevents false-positive label response in the microbial growth detection, compared to previously on package labels using BCG as a single
indicator (27). Thus, the dual indicators label gives a reliable response to the increase of the bacterial populations in the fish sample at both temperatures. Furthermore, the color changes of the on-package dual indicators label are a simple and effective active label for evaluating microbial counts roughly, where the fish spoilage was reached. Moreover, it can be stated that the dual indicators label can be employed to indicate the high microbial counts present in packaged fish, by their color change for visual detection that the fish was already spoiled.

**Texture and odor analysis**

The texture of fish sample measurements was conducted using a texture meter. The average texture softness values of triplicate measurements were depicted in Fig. 5a and Fig. 5b at room and chiller conditions respectively. According to these data, the dual indicators label response has a similar tendency with texture value. The freshness declines along with the incline texture softness of the fish samples (49). Hence, the dual indicators label can be employed to indicate the increase in texture softness of fish samples, as this value indicates deterioration of the fish sample.

The sensory evaluations of fish samples were conducted especially on a fish odor. The evaluations were conducted parallel with the dual indicators label response toward fish freshness status. The odor evaluation was conducted in the lab, with no treatment condition, as the label would be applied at home, shops, and others. The odor acceptance or odor score (%) of the fish samples evaluation is presented in Fig. 5c and Fig. 5d for room and chiller respectively. It is shown that the dual indicator label response is in a similar trend to odor evaluation (2), where the point of rejection of odor is 10 % in the room, and 0 % at the chiller, which were associated with the onset of detection of the dual indicators label color change (Fig. 5c and Fig. 5d) (34,49). This indication is shown by a color change of the dual indicators label for spoilage detection. Thus, the on-package dual indicators label was successfully applied for the packaged fish sample as a reliable active label for monitoring fish freshness statuses, such as fresh, medium, and spoilage (Fig. S2).

**CONCLUSIONS**

An on-package dual indicators label based on colorimetric paper-based pH sensors using BCP and BTB was developed for monitoring fish freshness. According to the results, the dual indicators label could be employed for determining fish freshness status and the correlation between the dual indicators label color change and the fish degradation over time is a similar trend, where the fish spoilage status could be determined visually (when BCP membrane change to purple and BTB membrane change to green-blue). The dual indicators
label reacts properly to the fish freshness status as their color change was met with
deterioration time point accordingly toward fish freshness status (fresh, medium fresh, and
spoilage). This is due to the moisture resistance property of the sensing label being improved
by simple adsorption of the reagent dyes which in turn, is allowing to prevent moisture from
interfering with the accuracy of the dual indicator label. Thus, the label can be feasibly
employed as a real-time active labeling device for effective fish freshness monitoring that can
be used as a simple tool in optimizing distribution and control of the fish product rotation
system, including reducing fish waste and loss.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest with this manuscript.

SUPPLEMENTARY MATERIALS
All supplementary materials are available at: www.ftb.com.hr.

AUTHORS’ CONTRIBUTION
Bambang Kuswandi contributed to the conception or design of the work and preparation
of the manuscript, Faridatul Hasanah did the data collection, Dwi Koko Pratoko contributed to
the data analysis and interpretation, Nia Kristiningrum contributed by performing the analysis.

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Fig. 1. Moisture absorption properties of plain filter paper (control), immobilized paper with BCP and BTB (a) a-b = Mean with the same alphabet are not significantly different (p < 0.05); and SEM images (b) of plain filter paper (i), and immobilized paper with BCP (ii) and BTB (iii).
Fig. 2. The rate of the dual indicators label change as the color response (mean RGB) towards spoiling fish at room (a) and chiller temperatures (b), and the pH values of packaged fish samples and the dual indicators label responses at room (c) and chiller (d) temperatures.
Fig. 3. The TVBN values of packaged fish samples and the dual indicators label responses at room (a) and chiller temperature (b), and The TVC value of packaged fish samples and the dual indicators label response at room (c) and chiller temperature (d)
Fig. 4. The texture values of packaged fish samples and the dual indicators label responses at room (a) and chiller temperature (b). The odor (%) of packaged fish samples and the dual indicators label responses at room (c) and chiller temperature (d)

SUPPLEMENTARY MATERIAL

Fig. S1. Design of dual indicators label for fish freshness
Fig. S2. The dual indicators label responses toward a different level of fish freshness, (a) fresh, (b) medium, and (c) spoilage