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Article

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Application of on-pack pH indicators to monitor freshness of modified atmospheric packaged raw beef

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Abstract

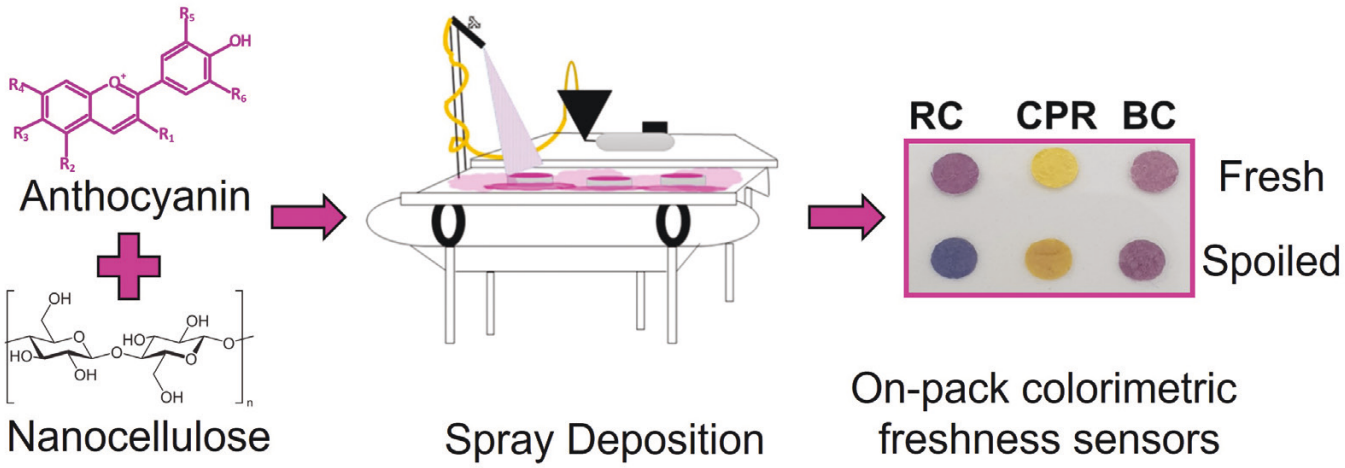
Objectives: To deveop on-pack visual indicators for the real-time monitoring of raw beef steaks in a modified atmospheric packaging (MAP).

Materials and Methods: Three indicators were prepared by spray deposition of a nanocellulose suspension (1.5%, in mass) with the desired concentration of the pH-sensitive indicators, either red cabbage (RC) extract, black carrot (BC) extract, or chlorophenol red (CPR). The responsiveness of the colorimetric pH indicators, assessed visually and by CIE-Lab quantitative analysis, to the freshness of raw beef steaks stored under MAP conditions at 4 °C or 20 °C, was analysed over 7 d.

Results: All the indicators showed a colour change for beef steak stored at 4 °C for 7 d that was noticeable with the naked eye and had a ΔE value>12. The sensitivity of the RC pH indicator was superior to that of the BC and CPR pH indicators. A study linking total microbial count (aerobic+*Escherichia coli*+coliform) and the quantitative colorimetric response of the indicators (ΔE) revealed a strong linear correlation.

Conclusions: The developed colorimetric pH indicators could be used to monitor the freshness of raw beef and as an alternative to the best-before date commonly used in pre-packaged meat.

Graphical Abstract



Keywords: Beef; modified atmospheric packaging; freshness indicator; food quality; anthocyanin.

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Introduction

Meat spoilage at a retail and consumer level occurs primarily as a result of contamination of the meat at the supplier's end, unsuitable packaging, or improper storage. The latter is often caused by a lack of consumer awareness regarding correct storage conditions (Khan, 2014). The freshness of beef degrades during storage due to microbial spoilage and associated biochemical reactions (Dave and Ghaly, 2011), and the use of the best-before date assists the consumer to make decisions related to the freshness and quality of pre-packed meat. However, food products can take many transit routes and experience different storage conditions, not all of which can be accurately controlled by a simple 'best-before' date (Frimpong *et al.*, 2014). This can lead to consumers either discarding food that is still fit for consumption, at an obvious economic and environmental cost, or eating food that is no longer safe. Importantly, the best-before date, noted at the time of packaging, only ensures the quality of the packed product if the required storage conditions are followed, and as a result, this date is not always accurate. An on-pack visual indicator confirming spoilage would eliminate consumers' confusion and reduce food waste.

Quantifying microbial growth on meat surfaces can be used to assess the quality of meat; however, these analyses are difficult to translate into an on-pack indicator, due to the diversity of possible microbes, their different product quality, and consumer health implications. Moreover, deterioration of product quality due to biochemical spoilage (enzymatic degradation and oxidation) is difficult to quantify using bacteriological methods (El Barbri *et al.*, 2008). A number of groups have utilized changes in the pH of raw meat (Edita *et al.*, 2018), and the subsequent volatile compounds formed, to determine meat freshness, resulting in the development of biosensors (Hernández-Cázares *et al.*, 2010; Bóka *et al.*, 2012; Singh *et al.*, 2018; Vedove *et al.*, 2021), electronic devices (that is, electronic nose, electronic tongue, and near infrared (NIR)) (Musatov *et al.*, 2010; Gil *et al.*, 2011; ElMasry *et al.*, 2012; Papadopoulou *et al.*, 2013; Wojnowski *et al.*, 2017), and colorimetric sensors (Alonso-Lomillo *et al.*, 2010; Li *et al.*, 2015; Liu *et al.*, 2015; Kuswandi and Nurfawaidi, 2017; Pei *et al.*, 2021). Although many of the abovementioned sensors provide accurate information, they are often expensive, challenging to interpret, or require special sample preparation or qualified personnel to interpret the sensors. As a result, they are not suitable for application in a retail store or home environment.

Freshness indicators, in general, provide information about product quality based on chemical changes or microbial growth within packaged food product (Lee and Shin, 2019; Hassoun *et al.*, 2022). Changes in these parameters generate an indicator response, usually a colour change, which is visually detectable and can easily be correlated with the freshness of food (Fuertes *et al.*, 2016). It is generally accepted that as meat spoils, the headspace environment's pH value increases; hence, on-pack pH indicators can be used to monitor freshness. It was recognized that an on-pack visual freshness indicator could be incorporated into meat stored in modified atmospheric packaging (MAP). As MAP of meat is conducted at the supplier, double handling is eliminated. Furthermore, the MAP film is non-permeable, thus volatile compounds produced over time will remain in the headspace. A number of research groups have reported the use of pH-sensitive dyes for meat freshness monitoring, with dyes immobilized on a range of solid substrates (Salinas *et al.*, 2014; Biji *et al.*, 2015; Shukla *et al.*, 2016; Kuswandi and Nurfawaidi, 2017; Priyadarshi *et al.*, 2021). However, testing of these dyes as indicators for packaged meat under MAP conditions has not been investigated, nor has a correlation study between indicators and microbial load to assess freshness. Although synthetic dyes have been used in a range of applications (Makote and Collinson, 1999; Pacquit *et al.*, 2007; Rukchon *et al.*, 2014; Kuswandi and Nurfawaidi, 2017; Zhang *et al.*, 2018; Magnaghi *et al.*, 2020), it has been recognized that consumers are concerned about their possible toxicity and, as a result, a number of natural dyes have been investigated. A recent review (Filho *et al.*, 2021) highlighted the positive aspects of anthocyanin dyes, in particular, for pH indication in food packaging. Since then, research into anthocyanin-based pH sensors has increased (for example, Ebrahimi *et al.*, 2022; Akhila *et al.*, 2023; Zeng *et al.*, 2023). However, these studies were conducted either on fish or chicken, or were performed *ex situ*. To date, no study has reported the *in situ* application of anthocyanin-based, pH-sensitive, on pack visual indicators of raw beef quality in MAP.

In this study, nanocellulose (NC) was chosen as the solid support for the dye due to its advantageous properties of biodegradability, recyclability, robustness, and thermal stability, as well as its high surface area and aspect ratio relative to cellulose fibres of longer length scales (Trache *et al.*, 2020), which improve the uptake and retention of dye molecules (Khatri *et al.*, 2014, 2016). In this paper, three colorimetric NC-based pH indicators based on natural red cabbage (RC) extract, black carrot (BC) extract, and the synthetic dye chlorophenol red (CPR) are presented. The indicator colour change was correlated with the surface microflora as a measure of the freshness of the packaged beef steaks.

Materials and Methods

Materials

Red cabbage extract was obtained from Universe of Science Inc. Black carrot extract and chlorophenol red were purchased from Botanical Cube Inc. (Xi'an, China) and Sigma-Aldrich (Sydney, Australia), respectively. The extracts and dye were obtained in powder form. Whatman® grade 42 filter paper was obtained from Merck (Bayswater, Australia). Industrial-grade nanocellulose fibres (microfibrillated cellulose, Celish KY-100S, 25% (in mass)) were obtained from DAICEL Chemical Industries Limited (Tokyo, Japan). 3M™ Petrifilms™ for aerobic counts and *Escherichia coli*/coliform were purchased from Thermo Fisher Scientific (Adelaide, Australia); CM0509 dehydrated buffered peptone water was purchased from Oxoid, Australia; plastic containers (black, 21.8 cm×15 cm×5.5 cm; for beef testing) were purchased from local Kmart; double-sided tape (Sellotape, Prestons, Australia), soaker pads (Sealed Air, Fawkner, Australia), and BioMedex self-sterilization pouches (135 mm×283 mm) were purchased from Alpha Medical Solutions (St Ives, Australia); Parafilm and MiniPax® absorbent packets (0.75 cm×1.06 cm) were purchased from Merck (Bayswater, Australia); 16×16″ Dimmable 70 LED Light Box was purchased from Ebay. CO_2 (20%) and 80% O_2 gas cylinder were purchased from Air Liquide (Melbourne, Australia). Hereford Porterhouse beef steaks,

from pasture-fed Hereford females aged<30 months and with the same best-before date (BBD), were purchased from Tasman Butchers (Clayton, Australia); circular acrylic discs (160 mm diameter) were cut from A2 acrylic sheet (420 mm×594 mm×4.5 mm; purchased from acrylicsonline.com.au) using a laser cutter.

Equipment

Digital orbital shaker (Heathrow Scientific, Vernon Hills, IL, USA), Memmert Incubator (*In Vitro* Technologies, Noble Park North, Australia), NU-S126-400E laminar hood (LAF Technologies, Bayswater North, Australia), Messmer disintegrator Model MK III C (Amsterdam, The Netherlands), Eurostar mixer (IKA, Staufen, Germany), and Wagner F 230 electric diaphragm pump (Melbourne, Australia) were used for the experiments described.

Preparation of dyed NC paper

The cellulose-based pH indicators discussed in this paper were developed based on the process described by Shanmugam *et al.* (2018). Dye-impregnated NC indicator paper was prepared from an NC suspension at 1.5% (in mass) by diluting the commercial NC (25% (in mass) solid content) with distilled water and the desired concentration of extract or dye (1% (mass concentration) of BC, 0.26% (mass concentration) of RC, and 0.01% (mass concentration) of CPR) was added. The dye containing NC suspension was mixed at 6000 r/min in a 0.5-kW Messmer disintegrator Model MK III C (Figure 1A). The pH of the disintegrated dye containing NC suspension was adjusted to pH 4 with 1 mol/L $HCl_{(aq)}$ while mixing with an IKA Eurostar overhead mixer (shear type) at 500 r/min. The pH-adjusted dyed NC suspension was sprayed onto circular acrylic plates (160 mm diameter) moving on a conveyor belt at 0.65 cm/s using a Wagner F230 electric diaphragm pump at a pressure of 100 bar (317 Wagner nozzle with a 0.38-mm orifice; Figure 1B). The distance from the nozzle tip to the surface of the acrylic plate was 30 cm. After spraying, the indicator paper on the plate was air dried for 48 h, peeled from the acrylic plate by hand, and stored in a desiccator with self-indicating silica gel at room temperature (Figure 1).

Indicator testing with beef

Porterhouse beef steaks (n=14, 250–300 g) were aseptically transferred from the commercial package to a plastic container (one steak per container) that contained one soaker pad and 17 silica gel packets attached along the length of the containers. The indicator paper was cut to a 0.6-cm diameter disc and double-sided tape was attached to the smooth side of the indicator paper so that it could be attached to the lid of the container. The container was then flushed with 80% O_2 and 20% CO_2 gas mixture for 30 s and the lid was closed and sealed with parafilm. Additional indicator paper discs were attached to the outside of the container (Figure 2). The containers were placed inside separate gasbags that were flushed with an 80% O_2 and 20% CO_2 gas mixture and sealed with a gas clip and Sellotape. The gasbags containing the plastic containers were stored in a refrigerator (4 °C) and two steaks were sampled daily for 7 d.

Colour analysis

The colour change response of the indicator papers was represented as colour difference (ΔE) values calculated from the CIE-Lab coordinates or L^*, a^*, b^* values according to established procedures (Ahmad *et al.*, 2019; Moradi *et al.*, 2019; Sun *et al.*, 2021). The CIELAB or CIE L^* a^* b^* system is a three-dimensional colour-space consisting of three axes. L^* is the lightness, a^* is the deviation towards green (negative values) or red (positive values), and b^* is the deviation towards blue (negative values) or yellow (positive values).

Colour analysis was conducted on the indicator papers in triplicate. L^*, a^*, and b^* were determined for the internal and external facing indicators on the lid of the plastic container containing the steaks. LAB values were obtained using a reflectance spectrometer (Ocean Optics HDX) including a

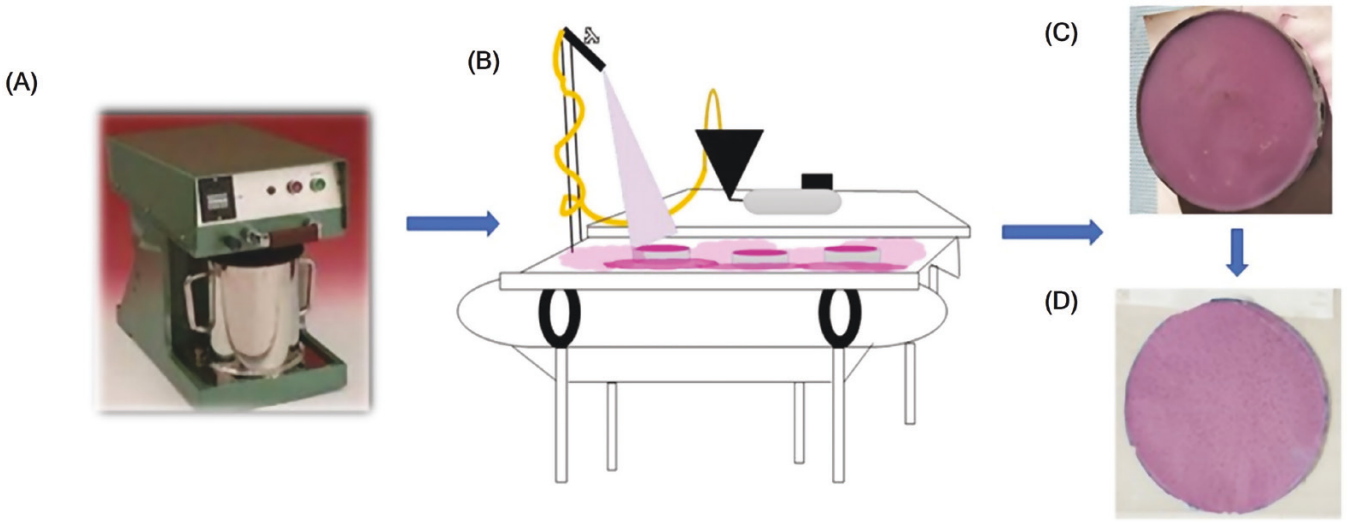

Figure 1. (A) The nanocellulose (NC) and indicator mixture is disintegrated to form a homogeneous suspension (1.5% (in mass) NC), (B) and (C) NC fibre with dye extract is sprayed on circular acrylic plate, example shown is with RC, (D) dried dyed NC paper.

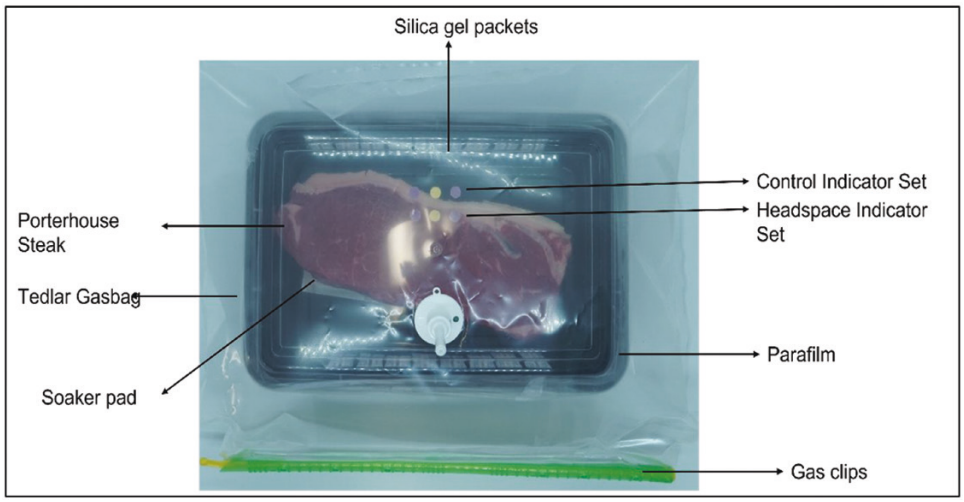

Figure 2. Diagram of the optimized simulated modified atmosphere package (MAP) used for testing the indicator response against raw beef steak.

DH-2000-L light source (200–1000 nm) and a UV–visible reflection probe (six illuminating fibres and one d=400 µm read fibre). Each indicator was placed horizontally on a black surface and the probe was placed at 45° with respect to the normal axis. All the measurements were taken in a dark environment (lights off). The reference of reflectance measurement was a Labsphere uncalibrated diffuse reflectance standard.

The colour change (ΔE) of the indicators inside the lid that were exposed to the volatiles released from the meat sample was determined in comparison to the external reference indicators (pH 4) on the outside of the lid using Equation 1.

$$\text{Colour difference } (\Delta E) = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2} \quad (1)$$

where the values of L_2*, a_2*, and b_2* are colour parameters of the indicators (attached inside the lid and exposed to the headspace) after interaction with the volatiles; and L_1*, a_1*, and b_1* are the colour parameters of the control indicators paper (pH 4) attached to the top of the plastic lids. A ΔE* greater than 5 can be detected by the naked eye, whereas values greater than 12 imply a complete colour difference, which is detectable even by untrained panellists (Tassanawat *et al.*, 2007).

Images of the pH indicators, prior to microbial analysis, were captured using a digital single-lens reflex camera (Sony α7iii camera body with a full-frame 90 mm f/2.8 macro lens) installed on top of a light chamber with a white background and under constant LED lighting to mimic retail lighting conditions. The images shown in the Supporting Information are a pictorial representation only and were not used to perform the colour analysis.

Microbial analysis

Determination of the aerobic bacterial count and *E. coli*/coliform growth on the surface of the porterhouse steak samples utilized modified methods developed by the Association of Official Analytical Chemists (AOAC; 990.12 (AOAC, 2015) and 998.08 (AOAC, 2019)). Daily, two steak samples were aseptically transferred to separate self-sterile bags, and 200 mL of sterile buffered peptone water (20 g of buffered peptone (powder) in 1 L of Milli- Q water, autoclaved for 20 min at 121 °C) was added and mixed using an orbital shaker (200 r/min for 2 min, and then 300 r/min for 1 min, at room temperature). Of this solution, 1 mL was diluted with buffered peptone water (9 mL), resulting in a 10^{-1} dilution. Serial dilution was done as required.

3M™ Petrifilms™ (n=3 for each dilution) were hydrated with 1 mL of sample of the appropriate dilution for the corresponding microbe of interest. The films were then incubated at 35 °C for 48 h. Following incubation, the colonies were photographed and counted using ImageJ®. The 3M™ Petrifilms™ that corresponded to dilutions producing≤150 colonies for aerobic microbes and ≤300 for *E. coli*/coliform were chosen based on the manufacturer's recommendation. Dilutions in the range of 10^{-1} to 10^{-3} were used for *E. coli*/coliform analysis and dilutions in the range of 10^{-3} to 10^{-5} were used for aerobic bacteria analysis. The logarithm of colony-forming units ($\log_{10}$ CFU/g) was calculated by taking the average number of colonies and multiplying it by the sample dilution factor, dividing by the total mass of the steak and subsequent $\log_{10}$ of this value.

Statistical analysis

Statistical analysis via one-way analysis of variance (ANOVA) was performed to determine the variation in microbial count and indicator response over the 7-d storage period. Two technical duplicates from each steak were used for microbial analysis and CIE lab values were used for indicator response calculations on the required day. Microbial data were analysed in technical triplicates and ΔE* values were measured from the two steaks each day and the measurements of the microbial analysis and indicator responses were averaged and were reported as the mean and the standard error of the mean. A mixed model of analysis of variance was performed on the three different indicator responses (RC, BC, and CPR) with respect to the microbial count throughout the 7-d period and between samples using one-way ANOVA. When the ANOVA revealed a significant difference in the indicator response against microbial counts over the storage period, as a post-hoc analysis, the Tukey Kramer multiple comparison test was applied. In all cases, the level of significance was set at P<0.05. The statistical

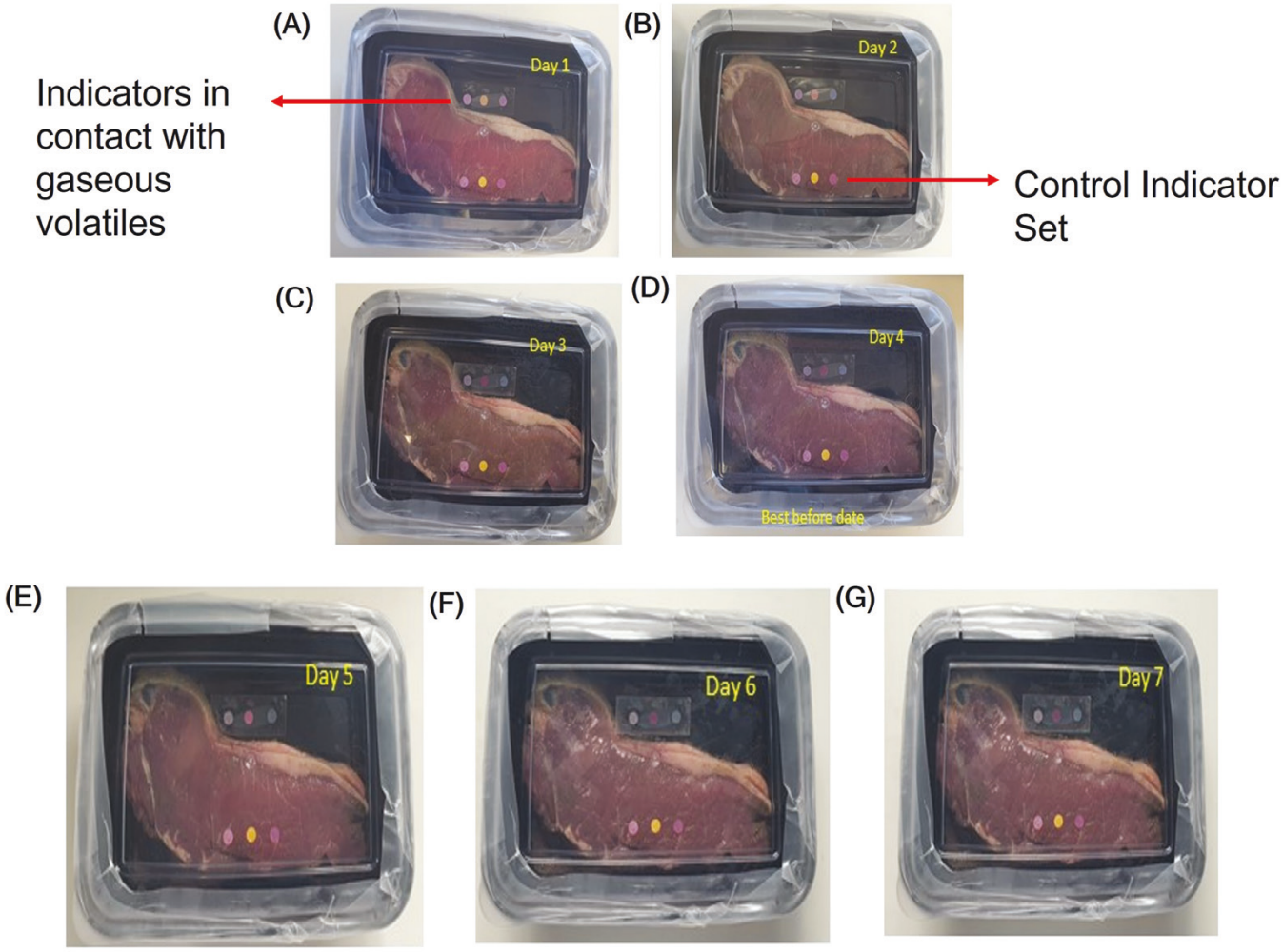

Figure 3. On-pack indicator response against raw beef steak stored at 20 °C for 7 d.

data of total microbes versus days and total microbes versus ΔE^* are shown in Tables S3 and S4.

Results and Discussion

Optimisation of simulated MAP and indicator response

Red cabbage and black carrot extracts were chosen due to their visual colour change within the desired pH range (Figure S1; Moradi *et al.*, 2019; Abedi-Firoozjah *et al.*, 2022). Beef is considered fresh and consumable at pH 5.4–5.8 and spoils above pH 6.4 (Węglarz, 2010). Chlorophenol red, a synthetic dye, changes colour in the presence of volatile compounds from meat (Magnaghi *et al.*, 2020). Although not currently approved for food use, its inclusion in this study enabled the performance and sensitivity of natural dye-based indicators to be compared with those of this synthetic dye. The indicators were manufactured by spraying the NC–dye suspension onto acrylic plates as described in the Materials and Methods section.

The black containers selected were chosen to ensure that the steaks were always horizontal during the experiment, the headspace above the steaks was no more than 3 cm, similar to commercial MAP, and their colour, which allowed visual changes in the steak and indicators to be readily observed. Indicator discs, three in total, were adhered to the inside and the outside of the lid of the container (Figure 3), with the outside indicator serving as the reference (control). The indicator disc had different textures on its two surfaces, as a result of the spray deposition technique. The side with greater surface area, exposed to air during the manufacturing process, was positioned so that it was exposed to volatile molecules in the headspace. With this setup, preliminary beef samples were prepared to test the efficiency and sensitivity of the indicators at 4 °C and room temperature (RT, 20–22 °C) for 7 d (Figure 3). At RT, not unexpectedly, all the indicators began to show a visible colour change within 48 h (Figure 3), with no further changes in colour after 72 h, suggesting saturation of the indicators. Over this time, due to the basic headspace environment, the pH changed from pink to blue (RC and BC) and from yellow to magenta (CPR). The meat samples were visually spoiled after 72 h at RT.

At 4 °C, a build-up of condensation within the simulated MAP was observed. To mitigate this issue, silica gel packs were added to the setup, reducing water condensation to a negligible level. Inconsistency of the indicator's colour change between the samples indicated that the loss of volatiles occurred

during storage. Therefore, secondary containment (a Tedlar gasbag also filled with the MAP mixture of O_2 and CO_2 gas) was applied to ensure that volatiles were retained, and the MAP environment was maintained around the sample (Figure 2). It is recognized that this setup varies from that of the commercial MAP, however, this study aimed to show the potential of the developed on-pack indicators in a simulated MAP environment.

With this optimized simulated MAP containment, 14 beef samples were prepared and stored at 4 °C. Two samples per day were analysed each day for colour change of the indicators and the enumeration of microbials over a 7-d period. The change in colour of the indicators was quantified by determination of colour change (ΔE); ΔE values larger than 5 can typically be detected by the naked eye, whereas values greater than 12 imply a colour difference that is detectable even by untrained panellists (Tassanawat *et al.*, 2007). The colour change (ΔE) of an experimental sample relative to the reference (control) indicator at pH 4 was determined used Equation 1.

The change in colour observed from pink to blue for both RC and BC indicators and yellow to magenta for CPR indicator was indicative of spoilage with the colorimetric response at 4 °C, directly correlating with the surface microbial count, as further discussed below. The ΔE values of the indicator response to the headspace volatiles inside the packages over the 7-d study period are given in Table S1 and Table S2. It should be noted that CIELab analysis was performed on the indicators themselves and not the images reproduced in the Supporting Information. The colour in digital images can be affected by many factors including screen brightness, image processing parameters, and camera settings, among others, which may lead to misinterpretation of data.

Microbial growth studies

The microbial aerobic plate count (APC) as well as the *E. coli* and coliform bacteria levels on the surface of the porterhouse steaks were determined. APC provides an enumeration of the total aerobic bacterial population, with a higher APC indicating poor quality and reduced shelf life (Kim and Yim, 2016). Some strains of *E. coli* bacteria are pathogenic and cause infection and food poisoning in humans (Harlia, 2017). Coliform bacteria are an indicator of disease-causing bacteria and the sanitary quality of food (Costa *et al.*, 2008).

From an industrial perspective, standard microbial testing is often done by subsampling of the meat (Kuswandi and Nurfawaidi, 2017; Sobhan *et al.*, 2021) and a total bacterial count of 7 log CFU/g is the approximate point at which red meat is considered to be spoiled or unacceptable (Dainty and Mackey, 1992). Many countries recommend or regulate that the APC level of raw beef be≤5–7 log CFU/g and that of *E. coli* be≤2–4 log CFU/g (Kim *et al.*, 2018). For raw beef, microbial spoilage begins when microbial levels reach 6–7 log CFU/g, and the meat starts to smell putrid as a result of free amino acid consumption of approximately 9 log CFU/g (Ercolini *et al.*, 2006). Another common measure of microbial activity is the total viable count (TVC), which provides a quantitative estimate of microorganisms such as yeast, mould, and bacteria in the sample (Jenkins and Maddocks, 2019). According to international trade, when the TVC exceeds 6 log CFU/g, meat is considered to be poor-quality and unfit for consumption (Ercolini *et al.*, 2009).

In this study, the choice was made to sample the entire surface of the steak, rather than subsampling. As the whole surface of the steak was responsible for the production of volatile compounds, and hence the change in the pH of the headspace, it was recognized that subsampling would not be representative of the microbial environment that facilitated indicator colour change. The response of ΔE versus log CFU/g is shown in Figures 4–6, images of the 3M™ Petrifilms™ and colorimetric responses of the indicators are reproduced in Figures S1–S8. In all cases, negligible *E. coli* colonies (<20) were observed. Pleasingly, levels of coliform and microbial aerobic plate count were correlated with the indicator response (Figures 4 and 5). As the *E. coli* microbial count was observed to be minimal in all cases, it was concluded that the indicator response was due to pH headspace changes as a result of the increasing coliform microbial count. Additionally, the increase in coliform microbial count had a concomitant increase in the ΔE values for all indicators, with the exception of one data point.

A similar trend to that described above was observed with aerobic microbial growth vs the ΔE value; that is, as the growth increased so did the indicator response (Figure 5). Although the values of ≤5–7 log CFU/g fall within the reported acceptable limits, these reports are based on subsample analysis and not an entire steak. This further indicates that subsampling approaches are not representative of the microflora present

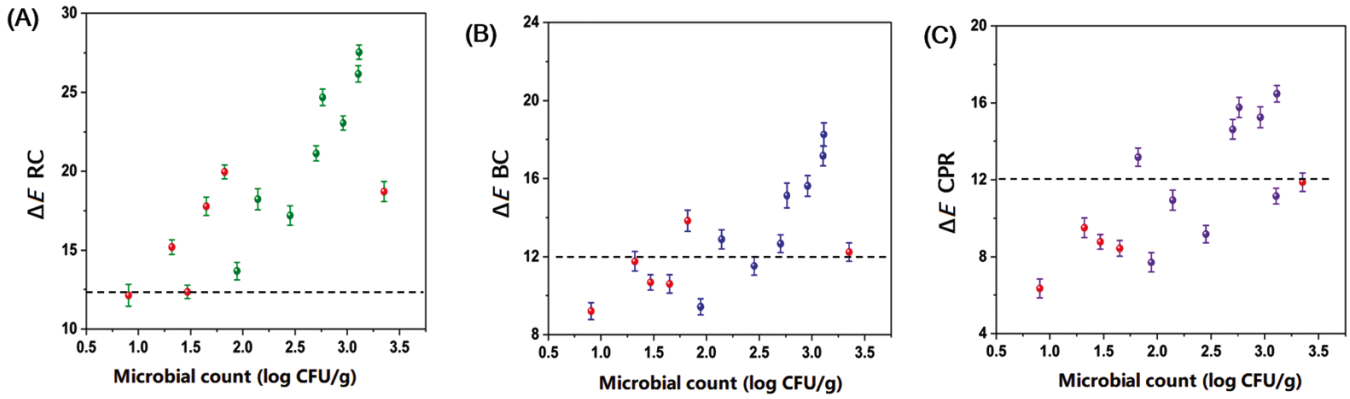

Figure 4. ΔE response of indicator versus log CFU/g for coliform (data from 10^{-3} dilution). Data outside the recommended colony count are shown in red. (A) Red cabbage (RC), (B) black carrot (BC), and (C) chlorophenol red (CPR). The dotted line at ΔE of 12 indicates that the colour change would be observed by the consumer.

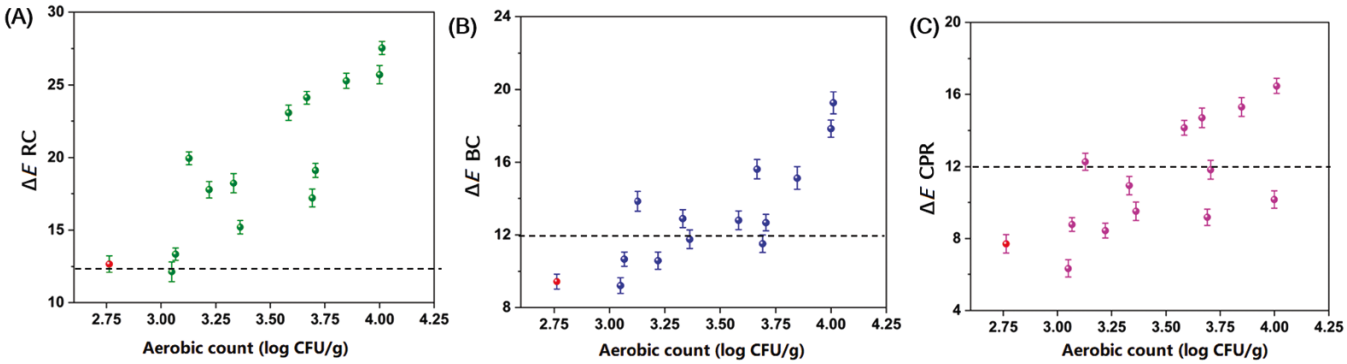

Figure 5. Δ*E* response of indicator versus log CFU/g for aerobic count (data from 10^{-4} dilution). Data outside the recommended colony count are shown in red. (A) Red cabbage (RC), (B) black carrot (BC), and (C) chlorophenol red (CPR). The dotted line at Δ*E* of 12 indicates that the colour change would be observed by the consumer.

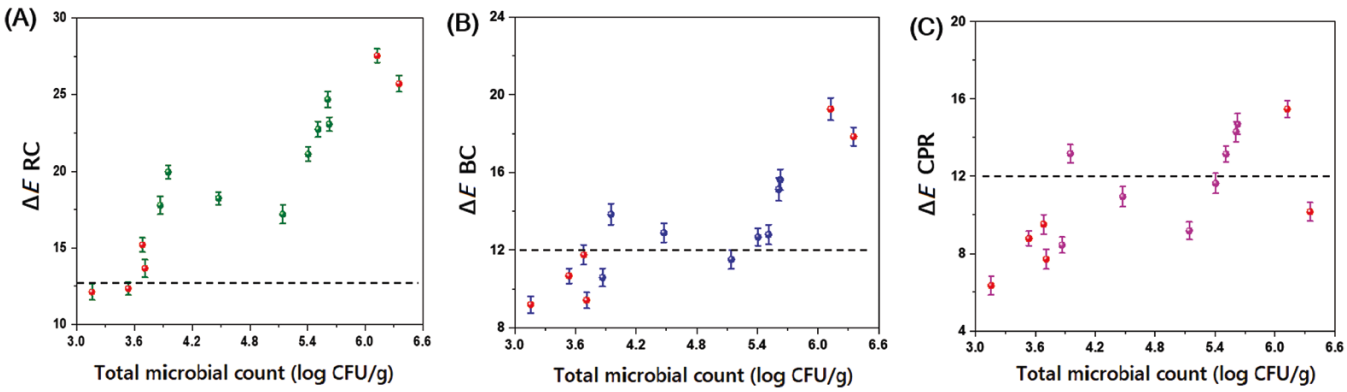

Figure 6. Δ*E* response of indicator versus log CFU/g for total microbial count (*E. coli*, coliform and aerobic count, data from 10^{-3} dilution); no *E. coli* colonies were observed at this concentration. (A) Red cabbage (RC), (B) black carrot (BC), and (C) chlorophenol red (CPR). The dotted line at Δ*E* of 12 indicates that the colour change would be observed by the consumer.

on the entire steak surface. It is worth noting that it is known that an increase in microbial aerobic plate count correlates with an increase in the pH value of the headspace gaseous mixture, supporting the concept that increased microbial activity has led to indicator colour change.

To correlate indicator response to the total surface microbial count, the sum of the log CFU/g microbial count for aerobic, coliform, and *E. coli* was determined by plotting log CFU/g versus Δ*E* (Figure 6). A total microbial count of 6.2 log CFU/g corresponded to highly discoloured steaks and a quantitative visual colour change in the indicators (Table S1 and Table S2). At a total microbial count of 6.2 log CFU/g, the Δ*E* values were greater than 12 for all indicators, indicating a clear colorimetric response, thus confirming that the pH is suitable to monitor the freshness of meat. Furthermore, drawing from the correlation between the observable colour change to the naked eye and microbial load, it can be inferred that these sensors have the capability to distinguish beef freshness status across three categories: fresh (CFU/g of 3–4), medium fresh (CFU/g of 4–6), and spoiled (CFU/g greater than 6).

In terms of indicator performance, the results of this study revealed that the RC indicator is the most sensitive to pH change during beef spoilage at 4 °C under MAP conditions (Figures S1–S7). It was noted that the BC and CPR indicators were less sensitive than the RC indicator, with a higher concentration of volatiles required to elicit a visible colour change. Although it was recognized that the synthetic CPR dye is not approved for food use, it was incorporated for its known response to pH changes; however, leaching from the NC was observed (Figure S7), and as a result, further studies

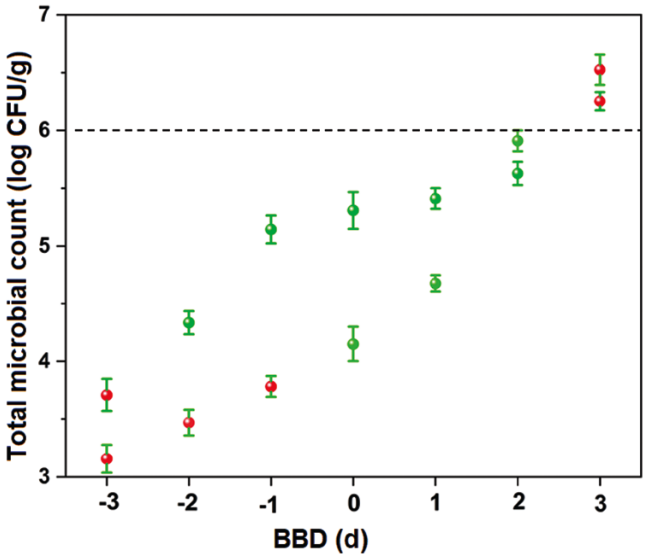

Figure 7. Total microbial count in the beef sample stored in modified atmosphere packaging at 4 °C for 7 d. All the points above the dotted line are above the acceptable limit of the total microbial count of 6.0 log CFU/g.

Figure 8. Example of on-pack labels that could be applied to modified atmosphere packaging for real-time quality indication and direct consumer interpretation.

would need to be performed if this indicator is to be used in a commercial setting. An additional measure may be the inclusion of a gas-permeable but water-impermeable membrane to protect the sensor from moisture.

The colour change of the RC indicator was the most prominent on day 5 of storage and the BC and CPR indicators showed a distinct colour change on day 7 (3 d after the BBD, 7.4 log CFU/g). The ΔE values of the indicator response to the headspace volatiles throughout the study are given in Table S1 and Table S2. Interestingly, different microbial load among steaks were detected on the same day with the same BBD. A possible explanation could be that the steaks had different levels of contamination at the start of the study, which further highlights the need for an on-pack indicator.

The above correlations clearly show that the indicator response is representative of the microbial count and that the indicators developed can be utilized to visually monitor the freshness of beef *in situ* in simulated commercial MAP. As the indicator colour correlates to log CFU/g, it follows from the data presented in Figure 7 that the current indicators could be incorporated into on-pack labels to replace the printed best before date on raw beef in commercial MAP, indicating the real-time quality of the product for direct consumer interpretation (Figure 8). In addition, the indicators could be used in the upstream supply chain to track product freshness during transportation and delivery to retail outlets.

Conclusions

Three pH-sensitive paper-based indicators were developed to determine the freshness of raw beef steaks under MAP conditions. All the indicators were shown to be potentially suitable for real-time monitoring of beef freshness at room temperature. They showed an accurate response to spoiled beef (stored at room temperature) with an intense colour change (pink to bluish-purple for RC and BC; yellow to magenta for CPR). At 4 °C (refrigerated temperature), the RC indicator showed a more accurate colorimetric response to beef freshness and surface microbial count when compared to BC and CPR. A strong correlation between microbial count and indicator colour change further validated the indicator responses. Therefore, with some modification, both CPR and BC indicators could be applied as an on-pack indicator of freshness for real-time monitoring of different food products under refrigerated conditions. Significant differences in microbial count across samples over the 7-d storage period were not observed. When the different indicator responses against the total microbial count at a particular dilution were statistically different, further post-hoc analysis using the Tukey Kramer multiple comparison tests was performed to identify the specific pair(s) of groups that caused the difference via one-way ANOVA test was performed. It was found that the indicator response between RC and BC to microbial count significantly differed, leading to change in headspace pH. Similar statistical differences were also observed for the comparison of indicator response between RC and CPR, whereas the response of BC and CPR to changes in headspace pH was not significantly different. The statistical results complement the visual change observed in the indicators, further validating the accuracy of their response to microbial growth and headspace pH change. These results confirm that the indicator made from red cabbage (RC) could be potentially used as a freshness indicator for raw beef inside MAP packaging. This study correlated the colorimetric change in the indicators with microbial count under simulated MAP conditions. However, it is important to note that these indicators have the potential to be utilized in alternate packaging methods, provided that using a non-permeable film to ensure that volatile compounds are retained in the headspace.

Supplementary Material

Supplementary material is available at *Food Quality and Safety* online.

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Author Contributions

Debarati Bhadury: Conceptualization, methodology, validation, formal analysis, investigation, visualization, and writing original draft; Humayun Nadeem: Methodology and investigation; Maoqi Lin: Methodology and investigation;

Jennifer M. Dyson: Conceptualization, resources, methodology, investigation, and review and editing; Kellie L. Tuck: Conceptualization, resources, review and editing, supervision, project administration, and funding acquisition; Joanne Tanner: Conceptualization, resources, review and editing, supervision, project administration, and funding acquisition.

Conflict of Interest

The authors declare no conflict of interest.

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