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Recent developments in colorimetric and optical indicators stimulated by volatile base nitrogen to monitor seafood freshness

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Abstract

Meat spoilage could cause food waste and also serious foodborne disease because of the specific organisms and their metabolites. Therefore, it is important to monitor the freshness of meat in the supply chain. In most cases, the spoilage of meat has been determined by the content of volatile base nitrogen, adenosine triphosphate or the total viable count. However, these destructive detection methods are tedious and time consuming. Hence, it is highly desirable to develop easily operated and non-destructive sensing technologies for real-time detection of meat spoilage. A lot of freshness indicators have emerged up to now. Colorimetric indicators are particularly beneficial to accompany use-by dates in food packaging as real-time indicators because they produce visible color changes to the naked eye, which can be easily understood by consumers and non-specialists.
Numerous studies have considered the colorimetric indicators to monitor meat freshness in recent years. This paper mainly focused on colorimetric indicators stimulated by volatile base nitrogen and used in monitoring meat freshness. The most traditional materials used in these indicators are also discussed. The challenges and opportunities in the systems are introduced in this context, as well based on the published literature.

1. Introduction

Packaging is an indispensable system of preparing foods for transport, distribution, storage, retailing and even end-use. It serves as the most important protection layer to prevent contamination and mechanical damage, as well as to prolong their storage life. With the developing of globalization and urbanization, consumers preference for more fresh and safe food products is on the rise, while the traditional packaging cannot meet the consumers demands. Also, the world population is projected to increase by almost one billion people within the next decade to reach 9.6 billion by 2050 (Poyatos-Racionero, Ros-Lis, Vivancos, & Martínez-Máñez, 2018). Such a high population implies that the consumption of food will corresponding grow. But the annual food waste generation has been estimated as around 1.3 billion tons according to FAO (FAO SAVE FOOD initiative). In this background, advances in packaging technology are essential to ensure food safety, avoid food spoilage to reduce food waste and satisfy consumer expectations.

Modified atmosphere packaging (MAP), which incorporates gas absorbing/emitting materials, antimicrobial packaging and controlled release packaging, has been established to address the above challenges. These new packaging systems are called active packaging (AP). AP is a promising innovation that has the potential to change in response to its environment and maintain the quality of packaged food (Kuswandi & Jumina, 2020). Despite major developments in packaging systems, the principles of packaging are mostly limited to enhancement of the protection without delivering the information or traceability of the products, which compels the development of packaging from a protector to an intelligent communicator. The evolution of intelligent packaging (IP) evolution is required at this time.

IP is capable of detecting, sensing, recording, tracing, or communicating information about the state of the product during the whole food chain (Yam, Takhirstov, & Miltz, 2005). It not only provides the information about the food itself, but the history of the surrounding environment, such as storage conditions, microbial growth, headspace gases, best use date and other relevant
information. Therefore, IP represents a big step in food packaging. Although we are still far away from achieving the final goal of having a generic intelligent packaging that will enable food safety and quality monitoring on a commercial scale, many companies have started to look into the possible use of indicators in food packaging. It was forecasted that IP will experience faster growth at a double-digit rate and will reach $24650 million in 2021 (Quezada, 2013).

In principle, IP is designed to at least have one indicator or device to sense the condition of the packed food or environment and inform people in the supply chain by an early visual warning (Realini & Marcos, 2014). Therefore, the indicator serves as the critical indentation and communication tool. It could respond to temperature (S. Choi, et al., 2020), time (J. U. Kim, et al., 2016), pathogens (Massad-Ivanir, et al., 2016), pH value (X. Zhang, et al., 2019) and gases (Meng, Lee, Kang, & Ko, 2015) by changing its physical appearance. There has been extensive research on the development indicators, and some of commercial indicators available on the IP market are shown in Table 1.

| Table 1 | Examples of commercial applications available on the IP market |
|-----------------|-----------------|-----------------|
| Commercial indicators | Name | Supplier |
| Time-Temperature indicators | 3M Monitor Mask™ | 3M Company |
| | Time strip® | Timestrip Plc |
| | Fresh-Check | Temptime Corp. |
| | Checkpoint | Vitsab |
| | Fresh Code® | Varvode Ltd. |
| | TopCryo® | TRACEO |
| Freshness indicators | SensorQ® | DSM NV and Food Quality Sensor |
| | RipeSense | RipeSense |
| | Fresh Tag® | COX Technologies Ltd |
| Radio frequency identification | Temptrip | Temptrip LLC |
| | Easy2log® | CAEN RFID Sri |
| | Intelligent Box | Mondi |
| | CS8304 | Convergence Systems Ltd |
| Integrity indicators | Tell-Tab | IMPAK |
Freshness indicators are such devices that directly indicate the deterioration or loss of freshness of packaged goods by reacting with the metabolites generated in the fresh food products as a result of microbial growth or metabolism (Realini, et al., 2014), such as glucose, lactic acid, ethanol, carbon dioxide, volatile nitrogen compounds, and sulfur derivatives (ABREU, CRUZ, & LOSADA). In this way, consumers could get early information of the food state by observing visual changes without opening the packing. In this way, such as system could prevent the consumption of unsafe food and consequently decrease the chances of food-borne illnesses in consumers, and prohibit potential outbreaks of illness (Yousefi, et al., 2019). Various freshness indicators have been described in the potential application of fish, shrimp, pork, kimchi (Meng, et al., 2015), fresh-cut green pepper (Ranjitha, Sudhakar Rao, Shivashankara, & Roy, 2015), guava (Kuswandi, Maryska, Jayus, Abdullah, & Heng, 2013), and milk (Weston, Kuchel, Ciftci, Boyer, & Chandrawati, 2020) etc. (Guo, et al., 2020). Due to meat’s high content of protein, enzymes and bacteria, it is extremely easy to decay; therefore, it has attracted most of study on the development of meat freshness indicators, as shown in Fig. 1. According to the statistics in Web of Science, we searched the key words of "freshness indicators", and there were 670 publications in total about freshness indicator from 1990 to 2020. Out of that same group, there were 182 papers that also used the term "meat", as shown in Fig. 1. An increasing trend was observed in the study of freshness indicators until 2019. And the frequency of the terms freshness indicator used in meat freshness reached up to 40% of articles about freshness indicators in 2017, and basically it maintained a ratio of 30% after 2017. The reported meat freshness indicators function by sensing the change of texture, bacteria (Escherichia coli), adenosine triphosphate (ATP), ATP decomposition (hypoxanthine) and volatile basic nitrogen (TVBN) (L. Wu, Pu, & Sun, 2019).
Adenosine triphosphate (ATP), the major carrier of chemical energy in living species, has been specially used as a stimulus for food quality control. Fluorescence sensors developed by graphene oxide (GO) and FAM (carboxy fluorescein)-labeled ssDNA (DNA-F) were employed for ATP detection in a wide range; such a system provided a satisfying result in the meat freshness evaluation (Z. Liu, et al., 2019). Since ATP completely disappears within 24 h after slaughter, it more often has been used in early detection (Watabe, Ushio, Iwamoto, Kamal, & Hashimoto, 1989). The K value was determined after ATP break down. It is the ratio of the sum of the concentrations of hypoxanthine over the sum of the concentrations of ATP and its decomposition products. It was found to be accurate for evaluating fish freshness, especially for edible fish at a level where spoilage is not so serious (Hamada-Sato, Usui, Kobayashi, Imada, & Watanabe, 2005).

After the decomposition of ATP, proteins are easily spoiled by the contamination of bacteria, including pseudomonas and lactic acid, which will cause the decarboxylation of amino acids, and further result in the production of biogenic amines such as tyramine, trimethylamine, 2-phenylethylamine, dimethylamine, putrescine, spermine, spermidine, tryptamine and agmatine. The volatile amines trimethylamine ammonia, and dimethylamine comprise TVBN, which are the characteristic substances responsible for meats. Hence, the TVBN content is usually recognized as a standard to determine sensorial quality. It is often measured by steam distillation method or Conway's micro-diffusion method. Both of the two methods need lengthy preparation: TVBN was first distilled into an alkaline solution, and then titrated with standard acid to calculate the content. Therefore, there have been increasing studies trying to construct indicators to sense the increasing
content of TVBN to further determine the freshness of meat. Electrochemical indicators, pH indicators, fluorescent indicators, and electronic noses are used for detecting TVBN. But color change is the most direct signal. Therefore, in this review, we will provide an up-to-date information on the colorimetric meat freshness indicators by detecting TVBN and analyzing the indicating materials or structures, which are directly incorporated into polymer systems such as film, sensor array as well as print ink. Current challenges and future directions for fresh indicators will also put forward.

2. Categories of colorimetric indicators in detecting TVBN

2.1 pH indicators

In an enclosed food package, as the concentration of TVBN increases, the pH value of inner environment will accordingly increase. From this perspective, pH indicator was designed by incorporating pH-sensitive dyes to sense the pH changes via an obvious color change. Typically, the color change of a pH-indicator is due to the protonation or deprotonation of a single class of either a carboxylic acid function or an amine group (Tamura, Abe, Ito, & Maeda, 1996). When only a single type of dissociable group is present, then one can expect a shift in composition from about 10% dissociated to 90% dissociated to take place within two units of pH. A broader pH range of color change can be expected in cases where more than one dissociable group is present.

It was expected that the color change would well correspond to the freshness level of meat, so that the consumers could get the freshness information by the color change, as shown in Fig. 2(a). The color parameters of indicators were measured by a portable colorimeter. The parameters L (lightness), a (redness-greenness), and b (yellowness-blueness) were used to evaluate the color difference ($\Delta E$). In theory, a value of $\Delta E$ greater than 5 can be easily detected by the human eye when viewing colored item on a label, while an apparent difference can be noticed even by untrained panelists when the values are greater than 12 (Prietto, et al., 2017). Smaller differences, such a $\Delta E$ value of 2, can be observed under ideal cases in which a large, uniform “test” specimen is immediately beside a “standard” color solid under ideal lighting conditions, (Wood, et al., 1989). As reviewed, a variety of pH sensitive dyes were introduced into pH indicators. Since it can be detected directly using the naked eye, pH indicators have been promisingly used as an on-package label for monitoring meat freshness.

2.1.1 Synthetic pH dyes
Representative acid/base indicators, such as phenolphthalein (Fleischmann, Cheng, Tabatabai, & Ritter, 2012), bromocresol blue (Shukla, Kandeepan, & Vishnuraj, 2015), bromocresol purple (Gavrilenko, Saranchina, Sukhanov, & Fedan, 2018) and methyl orange (Musso, Salgado, & Mauri, 2016) were first employed in pH indicators. All the synthetic dyes showed visual color differences, but their sensitivity was different. Incorporated phenolphthalein indicator turned red when the pH values were about 8 or higher. Bromocresol blue and purple indicator both were faint yellow at pH=3, and with the pH increasing to 6, their color turned blue and purple, respectively. As reviewed, in fresh meat the TVBN is around 6 mg/100 g or even less. The rejection limit of TVB-N for aquatic products is 20 mg/100 g according to the Chinese standard (GB 2733-2015). Corresponded to the TVBN, the initial pH of fresh meat is around 5.61 or less and a pH greater than 6.23 is indicative of the spoilage of meat. The indicators are known as highly sensitive material toward acid-base reactions in the pH range pH 5.61 to 6.23. Therefore, the bromocresol blue and purple indicator pH indicators were found to be more effective to give consumer early warning for the spillage food. In order to obtain a more accurate indicator system for freshness monitoring, methyl red and bromocresol purple were used simultaneously as pH dyes (Kuswandi & Nurfawaidi, 2017), as shown in Fig. 2(b). This dual indicator is more sensitive due to easy eye-catching coloration, as well as the fact that the two dyes can provide confirmation of each other. The system was successfully used as the indicator for monitoring of beef freshness status. However, these carcinogenic dyes were directly mixed on filter paper without further immobilization. Although the indicator had no direct contact with the food, there was no solid chemical binding between the sensitive dyes and the matrix, which will allow the migration of dyes into food, consequently posing a threat to consumer health. In order to fix the dyes, cationic chemical modification was used. Quaternary ammonia modified cellulose could immobilize anionic bromocresol blue via electrostatic interaction (Cao, et al., 2019). The release kinetics of dyes into solvents were studied. It was found that there was no dye released into ethanol solvents. But there was no further experiment to determine whether the dye was present in the tested fish product. Apart from that, a three-layered structure was designed to prevent direct contact (G. Y. Lee & Shin, 2016), as shown in Fig. 2(c). In addition, encapsulation may be a good way to prevent the release, but it may also prevent the stimulants from contacting with the dyes, consequently prolonging the response time. A reported study about multi-dyes encapsulated mesoporous silica aerogel claimed
that it had a short response time in acidic medium (0.71 s) and basic medium (0.36 s) (Islam, et al., 2019). But there was no related test in food. Due to the safety awareness, effective ways still need to be explored to guarantee that there is no migration of sensitive dyes to the food.

Fig. 2 (a) The operating mechanism of freshness indicators, (b) a double dye indicator system (Kuswandi, et al., 2017); adapted with permission of Elsevier and (c) three layers indicators (G. Y. Lee, et al., 2016), redrawn with permission of Springer.

2.1.2 Natural dyes

Natural dyes gradually served as alternatives to synthetic dyes due to both legislative actions and consumer health concerns. Most natural dyes are polyphenols, which are widely present in plants. Anthocyanins are one of the most used natural dyes. Most leaves, flowers and fruits that have brilliant colors (blue, red, and purple) contain anthocyanins. Examples include purple sweet potato (I. Choi, Lee, Lacroix, & Han, 2017), Bauhinia blakeana (X. Zhang, Lu, & Chen, 2014), mulberry (Ma, Liang, Cao, & Wang, 2018), grape skin (Ma & Wang, 2016), rose (Guo, et al., 2020), red pitaya peel (X. Zhang, et al., 2019) and etc. The anthocyanins in different pH buffer solutions possessed obvious color differences, which can be observed by the naked eye, as shown in Table 2.
generally, they had a strong red color in the pH range of 2-3, and the solution color changed to pink around pH 5.0–5.5. as the pH increased to over 7, the color gradually changed to green. But some anthocyanins displayed distinguishable color changes in the pH range of 5.0-6.0 and 6.0-7.0, as reported for anthocyanins extracted from black chokeberry (Halász, et al., 2018) and rose (RAs) (Kang, et al., 2020). Such changes can be advantageous to indicate the freshness level of meat. The resulting RAs freshness indicator in monitoring shrimp gradually changed from purple to blue (18 h), dark green (24 h) and yellow (32 h). When the film was dark green, the TVBN level of shrimps reached 21 mg/100 g, demonstrating that the shrimps were seriously spoiled and no longer edible ("end-use "). Also, the blue color represented a "best use" date, which was useful to decrease food waste. Therefore, there were increasing studies try to enhance the responsive abilities, such

<table>
<thead>
<tr>
<th>Variety</th>
<th>Transition pH range</th>
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<tbody>
<tr>
<td></td>
<td>2.0</td>
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<tr>
<td>red cabbage extract</td>
<td></td>
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<tr>
<td>Lycium ruthenicum</td>
<td></td>
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<tr>
<td>jambolan</td>
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<tr>
<td>rose anthocyanins</td>
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<tr>
<td>alizarin</td>
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<tr>
<td>black bean extract</td>
<td></td>
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<tr>
<td>purple sweet potato</td>
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<tr>
<td>black carrot</td>
<td></td>
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<tr>
<td>Echium amoenum</td>
<td></td>
</tr>
<tr>
<td>black chokeberry</td>
<td></td>
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<tr>
<td>eggplant</td>
<td></td>
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<tr>
<td>Spirulina sp.</td>
<td></td>
</tr>
<tr>
<td>mulberry</td>
<td></td>
</tr>
<tr>
<td>mulberry+ZnO₂</td>
<td></td>
</tr>
<tr>
<td>rich black plum peel extract</td>
<td></td>
</tr>
<tr>
<td>rich black plum peel extract + TiO₂</td>
<td></td>
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</tbody>
</table>

Table 2 The color change of pH indicators prepared from plants dyes.
as RAs fresh indicators that include both a "best use" date and an "end use" date. Blended curcumin and anthocyanins were used to enrich the color change (H.-z. Chen, Zhang, Bhandari, & Yang, 2020). It was found that curcumin and anthocyanins at a ratio of 2:8 (v/v) could provide three different colors, which were assigned to the sign of freshness, medium freshness, and spoilage for packaged food. Also, there was a study committed to increase the specific surface area to enlarge the contact area to the surrounding pH environment by electrospinning (Guo, et al., 2020); meanwhile, carvacrol was incorporated to endow the indicator with antibacterial properties. The increased surface area was also effective to control the release of carvacrol. An aerogel also provides ultra-high surface area, so there is a need for research of aerogel usage in food freshness indicators.

Although interest in anthocyanins has intensified, anthocyanins are easily degraded by the effects of light, enzymes, oxygen, pH, and temperature. etc. (Liang, Zhang, & Jing, 2019). Their stabilities are critical for the application in freshness indicators. A large body of evidence has shown that the interactions between anthocyanins and polysaccharides played an important role in increasing the stability of anthocyanins, which is possibly governed by hydrogen bonding and electrostatic interactions (Jakobek, 2015; Jeewon Koh, Zhimin Xu, & Louise Wicker, 2020). In addition, several reviewed papers provided evidence that the interactions could also extend anthocyanins π-π conjugated systems and further enhance their colors (J. Koh, Z. Xu, & L. Wicker, 2020; Trouillas, et al., 2016). This could explain why most anthocyanins used in the freshness indicators were normally obtained by acid/ethanol extraction without further purification. The crude anthocyanins contained polysaccharide or phenolic acid, which was beneficial to enhance their stabilities. It would likewise be understandable that crushed grape skin (Chi, et al., 2020) or frozen black berry powder (Nogueira, Soares, Cavasini, Fakhouri, & de Oliveira, 2019) have been directly used to prepare fresh indicators. Despite the fact that it is a convenient method, the dispersion of the powders needs to be firstly considered. There are also some other natural dyes with conjugated structure, such as chlorophyll (Veiga-Santos, Ditchfield, & Tadini, 2011), curcumin (Ma, Du, & Wang, 2017), alizarin (Parya Ezati & Rhim, 2020) and pigment from the microalga Spirulina (Goettems Kuntzler, et al., 2020), have been used in freshness indicator. Except for the above natural dyes, natural dyes can also be sourced from microbial fermentation without dependence on seasonal variations, which can be a problem with dyes from plants. Red
(L. Sun, Zhang, Qin, & Zhao, 2016), yellow (Dufossé & de Echanove, 2005), black (Pombeiro-Sponchiado, Sousa, Andrade, Lisboa, & Gonçalves, 2017) and blue pigments (Zhu, et al., 2020) have been produced from microbes. In addition, the blue pigments have been shown to possess purplish-red color at pH 6.0, and then to change to blue as the pH is increased to 7.0 (Zhu, et al., 2020). As a consequence, the blue pigments have potential use in freshness indicators. But the authors did not find any related applications in fresh indicator.

2.1.3 Polydiacetylenes

Polydiacetylenes (PDAs), representing a class of conjugated polymers, have been extensively explored as the stimuli-responsive colorimetric sensors (W. Lee, Lee, Kim, Lee, & Yoon, 2018). They are typically generated by topo-polymerization of organized diacetylene (DA) monomers under the treatment of UV light or γ-ray irradiation without chemical initiators or catalysts (Chanakul, Traiphol, Faisadcha, & Traiphol, 2014). It was found that PDAs have a blue color at $\lambda_{\text{max}}=640$ nm, as shown in Fig. 3a. A variety of stimuli induced a blue-to-red ($\lambda_{\text{max}}=550$ nm) color transition in the PDAs, such as temperature, pH, mechanical stress, solvents and ions, and all of that can be controlled by structural modification (W. Lee, et al., 2018). The interactions between the –COOH groups of PDA and ammonia could trigger a blue-to-red color change of the compound. So, Park et al. developed an intelligent film based on the PDAs monomers (10,12-pentacosadiynoic acid) and 6,8-heneicosadiynoic acid to detect vapor ammonia gas generated by food spoilage. In addition, PVA was used to address the limitation of rigid PDAs sensors. There were discernible colorimetric changes in real-time detection of beef and hairtail spoilage, which could be observed by the naked eye (Park, Lee, Cui, & Ahn, 2016). But the films needed 1.5 h to show a visible color change when exposed to at least 1000 ppm of ammonia at 25 °C, which was much longer than the curcumin-based film (Ma, et al., 2017). Thus, there has been research using cellulose nanocrystals (CNC) and chitosan to fabricate highly sensitive PDAs film, as shown in Fig. 3b (Nguyen, Naficy, McConchie, Dehghani, & Chandrawati, 2019). The color transition of the resulting indicator was noticeable after 30 min of exposure to 1000 ppm ammonia at -20 °C. Also, the indicator exhibited a distinctive blue-to-red color transition during the meat spoilage from room temperature to -20 °C. The wide range of operating temperature can be advantageous in practical applications compared with natural dyes. Further study needs to be conducted to optimize the freshness indicators according to the type of food, and to correlate the extent of color
transition with the level of spoilage. Besides, the PVA-PDAs indicator also undergoes non-fluorescent to fluorescent after reaction with ammonia gas. Therefore, PDAs have been extensively utilized for designing stimuli-responsive colorimetric and fluorometric sensors. PDAs were incorporated with imidazole and triazole derivatives to detect HCl gas (W. Lee, et al., 2018), and also engineered as milk freshness indicators (Weston, et al., 2020). Easy visualization of color change was obtained, so a system showing fluorescent changes exposed to food spoilage would represent a significant improvement, since fluorescent materials can sensitively differentiate guest species (Z. Zhang, Wang, & Chen, 2019). The visually fluorescent freshness indicators are discussed in detail later in this article (section 2.2).

Fig. 3 (a) Schematic representation of PDAs chemical structure with UV light and stimulation and (b) CNC/chitosan/PDA indicator fabrication according to reference (Nguyen, et al., 2019), reprinted with permission from Royal Society of Chemistry.

2.1.4 Nanoparticles

Nanoparticles can aggregate or change their size when they were under stimulus, consequently causing a visible color change. Metallic nanoparticles, especially Au nanoparticles (AuNPs) were selected to design optical indicators. Fig.4(a, b) shows that the color of AuNPs changed from burgundy to blue/gray with the inducement of histamine (Lapenna, Dell’Aglio, Palazzo, & Mallardi, 2020). Such as system has potential to be applied to meat freshness indicator. Silver nanoparticles (AgNPs) embedded in bacterial cellulose were also designed to monitor meat
The size of AgNPs was dramatically modulated, thus showing a change in color from amber to gray in the presence of volatile amines compounds (Heli, Morales-Narvaez, Golmohammadi, Ajji, & Merkoci, 2016), as shown in Fig. 4(c-f). There were also zinc oxide (ZnO) and titanium dioxide (TiO$_2$) to enrich the color varieties, which will be discuss in later sections. The in-situ growth of noble metal nanoparticles provides a general method to prepare visible color readouts with high resolution.

Fig. 4 (a) Schematic representation of AuNPs and their color change stimulated by histamine (b) (Lapenna, et al., 2020), reprinted with permission from Elsevier. (c) UV–vis spectra of the nano plasmonic membranes before food spoilage exposure (Ctrl) and after food spoilage exposure. (d) Appearance of the nanoplasmonic membranes before/after food spoilage monitoring. (e) TEM micrographs, from left to right: control, fish spoilage and meat spoilage. (f) Size distribution of the AgNPs embedded in the nanopaper before/after food spoilage monitoring, the box plots show the median, 25th and 75th percentiles and the extreme values of the respective size distributions (5–95 percentile) (Heli, et al., 2016), reprinted with permission from Royal Society of Chemistry.

2.2 Fluorescent indicators

There have been lots of studies using fluorescent materials to sense the volatile ammonium compounds that emit colorimetric luminescence signals under particular excitation of wavelength. Therefore, fluorescent analysis also has immense potential for freshness indicators owing to its
remarkable advantages of high sensitivity, ease of operation, low cost and rapid result outputs. Organic fluorescent dyes and nanomaterials are classical fluorescent materials (Luo, et al., 2020). A fluorescent nanotube assembled from asymmetric perylene diimide molecules exhibited high sensitivity to amines and capable of monitoring the meat deterioration. But a time-dependent fluorescence quenching phenomenon was observed in the detection of pork, fish, shrimp and chicken within 4 days (Hu, Ma, Zhang, Che, & Zhao, 2015). In order to avoid aggregation-induced quenching, Alam et al. successfully applied a series of 1,2-dihydroquinoxaline derivatives (DQs) with aggregation-induced emission (AIE) characteristics for detecting ammonia vapor at a concentration of 690 ppb and effective to monitor sea food spoilage by visible color shown in Fig. 5(a) (Alam, et al., 2017). But this indicator had low cytotoxicity to cells. So, there was a study using quercetin to prepare an AIE composite sensor (T. He, et al., 2018). The quercetin-AIE sensor not only indicated the spoilage of salmon, but it also had antioxidant properties to prolong the shelf life of food compared with DQs. The natural fluorescent dyes incorporated multi-functional freshness indicator is promising in improving food storage and indicating food quality.

The ratiometric fluorescent systems also provide promising application in freshness indicators because they have two luminogens. Generally, one acts as the indicator of analyte and another as the internal reference to increase the detection accuracy and extent of color changes. A ratiometric fluorescent indicator was composed of fluorescent isothiocyanate and protoporphyrin, which were covalently bonded onto cellulose acetate chains (Jia, et al., 2019). The prepared fluorescent sensor exhibited a sensitive, color-responsive and linear response to ammonia within a wide range of 5.0 ppm to $2.5 \times 10^4$ ppm under 365 nm UV, also providing a high-contrast color change for real-time, visual, and accurate monitoring seafood freshness, as shown in Fig. 5(b).
Carbon dots and graphene oxide derived fluorescent sensors also can be used as versatile tools for food quality assessment. However, carbon dots generally have been found to display high selectivity for the recognition of NOx, aldehyde species (Pacquiao, de Luna, Thongsai, Kladsomboon, & Paoprasert, 2018) and metal after surface functionalization (B. Wu, Zhu, Dufresne, & Lin, 2019). Graphene oxide was developed for detecting adenosine triphosphate (ATP), further allowing its effective use for evaluating the freshness of meat samples. Also, there has been tremendous interest in fluorescence spectroscopy due to its relatively high sensitivity, low detection thresholds ($10^{-7}$–$10^{-9}$ g/mL) and non-destructive characteristics. Front face florescence spectra and fluorescence excitation-emission matrices have the potential for fingerprinting and real-time monitoring of meat quality (H. Liu, et al., 2019).

3 Forms of freshness indicators

Freshness indicators take many forms, including films, emulsions, printing ink and colorimetric sensor array in response to biogenic amines. In this section the preparation, along with response efficiency in food packaging are discussed.

3.1 Films

The research of freshness indicators has focused on films or labels derived from PVA, polysaccharides (cellulose, gum, chitosan and etc.) and proteins due to their degradable properties.
The reported pH-sensitive dyes were directly introduced on these film matrices through hydrogen bonding or ionic reactions. The polysaccharide was the most common matrix, because the hydrogen bonding between the polysaccharides and anthocyanins played an essential role in stabilizing anthocyanins, which is called the co-pigmentation effect (Trouillas, et al., 2016). The highest affinity was observed with pectin and anthocyanins, compared to other polysaccharides or other phenolics, possibly governed by electrostatic interaction and anthocyanins stacking (J. Koh, et al., 2020). Therefore, there have been numerous studies developing the pectin-based freshness indicator. As the research continued, the researchers found that hydrophilic performance of film was favored to adsorb water molecules. Sufficient water molecules adhering onto the film surface may directly react with the volatile basic nitrogen to NH₄⁺, and consequently accelerate the response. That would explain why deep eutectic solvents (DES) were used as additional plasticizers to optimize pH intelligent films (Pereira & Andrade, 2017). It is highly desirable to reduce the permeability of water vapors before being used as a food packaging material. Except for the hydrophilies, it was reported that porosity is effectively responsive to the stimulus due to the relatively larger specific surface area and being able to have a color change. As a consequence, cellulose nanocrystals (Ma, et al., 2016), electrospinning nano fiber mats (Fig. 6(a)) (Guo, et al., 2020) and nanoparticles titanium dioxide (Fig.6(b)) (X. Zhang, et al., 2019) were used to increase the sensitivity. It was found that they were effective to enrich the color variations. Biogenic amines, as small size targets, will pass through the nano-porous film; therefore, the pore size distribution would also affect the efficiency. In this perspective, aerogels would be a good option. Aerogels have been intensively used in detecting volatile organic compounds, but they haven’t been found in food freshness indicators (Dolai, et al., 2017).
Fig. 6 (a) Fabricating Zein (ZN)/Glycerol (GL)/Pullulan/Purple sweet potato extract (PSPE) indicator by electrospinning and their application in detecting pork freshness (Guo, et al., 2020). (b) TiO\textsubscript{2} nanoparticles enhanced Chitosan/black plum peel extract (BPPE) films and their color changes immersed in different buffer solutions (X. Zhang, et al., 2019). Both figures were reprinted with the permission of Elsevier.

Water-soluble conjugated polymers have been intensively studied for producing new colorimetric fluorescent films. While transferring to lower energy electron/energy acceptor sites over long distances, the excitation energy along the entire backbone of conjugated polymers resulted in an amplified fluorescence signal, and it made them ideal materials for sensors (H. N. Kim, Guo, Zhu, Yoon, & Tian, 2011). Moreover, for one luminogen, there is only a minor change of fluorescence intensity in most cases, and the limited sensitivity of human eyes to the change of fluorescence brightness might increase the experimental errors or even cause a failure in the naked-eye detection mode (Chow, Lam, & Wong, 2013). Therefore, the ratiometric fluorescent systems showed great potential (Wang, et al., 2011). However, the preparation of fluorescent conjugated polymers may use organic solvents, and the contamination and potential toxicity may hinder their application in food freshness indicators.

Fig. 7 (a) The schematic representation of electrochemical writing on hydrogels and the resulting written agar (AG)/anthocyanin (AH)/gellan gum (GG)/TiO\textsubscript{2} product (Zhai, et al., 2020). (b-c) Color response of written films in different time intervals and TVB-N values of the fish and their relative color change and images during the storage of 16 days at 25 °C (S. Wu, et al., 2018). Both figures were reprinted with permission of Elsevier.
Additionally, an electrochemical writing process was used to allow the printing of varied information on the intelligent films (Zhai, et al., 2020). The process is induced by hydrogen ions and hydroxyl ions generated from water electrolytic reactions (Fig. 7(a)). Hence, the process was conducted on hydrogels containing high contents of water before the film drying process. The writing is temporary on the hydrogel, but the pattern can be fixed when the hydrogel is dried (Jia, et al., 2019). It was reported that the written film retained color change sensitivity to ammonias, as shown in Fig. 7(b) (S. Wu, et al., 2018). In addition to the retainability of the written information, the stability of anthocyanin in the printing film was unexpectedly enhanced. Fig. 7(c) showed that the relative color changes (S) of the written chitosan/agarose/anthocyanin films were all lower than 5% after being stored at 25 °C and 0% and 75% RH for 16 Days. Besides, the S value of written film was lower than that of a blank film, which may be attributed to the low water content of the dried films, and this inhibited the hydration of anthocyanin and conserved the color (Lewis, 1995). These colorimetric films can be incorporated into entire packaging films, whereby the entire package can emit a color change or a signal. These films can also be placed strategically in the package in the form of sensor “chips” or “windows”, which can be visualized.

3.2 Colorimetric Sensor Array

Colorimetric sensor arrays utilize cross-responsive, chemically responsive dyes to generate a composite, olfactory-like response, which is unique to a given odorant and could be quantified by digital imaging (Xiao-wei, Xiao-bo, Ji-yong, Zhi-hua, & Jie-wen, 2018). Such a sensor usually contains responsive dyes and a solid support. The solid support general provides a uniform white background and does not react with the dyes. Also, it was reported that an accessible nano-porous structure would enhance the diffusion of analytes to chromophore (Xiao-wei, et al., 2016). Different from polysaccharide-based intelligent film, a hydrophobic substrate would prefer to reduce the interference effects with ambient humidity. As a consequence, the silica gel plate (Bordbar, Tashkhourian, & Hemmateenejad, 2018), titanium dioxide (Xiao-wei, et al., 2016) and hydrophobic porous membrane (Sen, Albarella, Carey, Kim, & McNamaraiii, 2008) have been the most commonly used plates. For accurate quantification, the color parameters (Red (R), Green (G) and Blue (B)) of sensor array image was then extracted by an image acquisition device and further processed by principle component analysis (PCA) and hierarchical cluster analysis (HCA) to quantify the result. Morsy et al. developed a silica gel array sensor composed of sixteen sensitive
dyes, which included the synthetic dyes (methyl red, Bromophenol blue, purple, green and etc.) and natural dyes (curcumin and alizarin) to monitor Atlantic salmon. (Morsy, et al., 2016) The resulting sensor array was capable of discriminating a variety of targets spontaneously, such as pH, TVBN and TBV. After calibration, the color change correlated well with changes in pH and TBA content compared with TVB-N at 4 °C.

With the technology development of smartphone, the color information could be captured by photographing the membranes using an app by analyzing the grey scale from the RGB space (Perez de Vargas-Sansalvador, et al., 2020). Fig. 8(a) showed that it could provide a quick test to determine the pork quality. A colorimetric geometric barcode sensor was designed based on the sensor array containing Nile red, Zinc Tetraphenylporphyrin and Methyl red, as shown in Fig. 8(b) (Y. Chen, et al., 2017). The fabricated sensor was attached on the surface of the meat or on the inner lining of the package. The status of the chicken would be obtained after taking a photo of the barcode sensor. The color information (RGB parameters) extracted by a custom app on smartphone would be compared with the original one printed on package to decide the fresh level of the meat. The constructed hand-held smart device was able to effectively indicate the aging status of chicken in two weeks under 5 °C and -5 °C. But it was difficult to differentiate the responses after three days in 20 °C, since the sensor response reached saturated.

Hyperspectral imaging (HSI) has the ability to capture more information than a normal camera. Each pixel of the captured image can be transformed to a "spectral feature". As a consequence, the meat information is isolated and transformed into specific wavelengths. Integrating colorimetric sensor array to fully evaluate the pork freshness have been studied by previous researchers (Li, Chen, Zhao, & Wu, 2015). Although the proposed method consisted of two independent systems, their resulting data (color RGB information and extracted wavelengths from HSI) were fused to improve accuracy and obtain more inferences. An optimum result was achieved with a correction coefficient of 0.932 in the monitor of pork freshness. Therefore, the application of colorimetric sensor arrays for monitoring food quality could also be improved in the future, given the technological development.
Fig. 8 (a) Data analysis and color measurement by Android app in cellphone to determine meat quality (Perez de Vargas-Sansalvador, et al., 2020). (b) A cellphone-based colorimetric sensor array used for determination of chicken freshness (Y. Chen, et al., 2017). The figures were reprinted with the permission of Elsevier.

3.3 Printing ink

Colorimetric dyes have been used as inks to directly print patterns. The discussed fluorescent isothiocyanate and protoporphyrin dyes in fluorescent indicators section were printed into a maple leaf (Jia, et al., 2019). And it didn't affect its fluorescent properties upon exposure to vapor amines. Printing makes the above prepared dyes very suitable for mass production. A time temperature indicator (Zabala, Castán, & Martínez, 2015) and carbon dioxide (Y. Zhang & Lim, 2016) indicator by printing technologies were reported. It was found that the solution properties of the ink, such as viscosity, surface tension and solvent volatility are crucial for manipulating the droplet formation and determining the ink-substrate compatibility. A thickener may be used to control the rheological properties.

With the development of three-dimensional (3D) printing, it has been widely applied in machinery, food and medicine industries due to the advantages of timesaving, refined shapes and easy operation. Food 3D printing provides a new frontier to make full use of by-products from fruits, fibers and vegetables. Potato residuals, lemon juice (Yang, Zhang, Bhandari, & Liu, 2018) and other components (Liu, Zhang, Bhandari, & Wang, 2017) have been printed into biscuit, cake, and cracks. Based on that, it is put forward that 4D printing could respond over time under environmental stimuli (such as temperature, water, pH, light or gases) (Le-Bail, Maniglia, & Le-Bail, 2020). A recent paper reported that a sample prepared by 4D printing of mashed potato and
purple sweet potato with spontaneous color change in pH condition due to the presence of anthocyanin rich in purple sweet potato, as shown in Fig. 9 (C. He, Zhang, & Guo, 2020). Thus, anthocyanins might be a promising ingredient for the 4D printing of food products in terms of spontaneous color change under pH stimuli condition, even used in the food decomposition stage. However, until now there are very few works focusing on 4D technologies for smart food indicators.

Fig. 9 Color changes images (a) and parameters (b) of mashed potatoes with different formulations over time after 4 D printing (C. He, et al., 2020), copyright 2020 Elsevier.

4 Conclusions and future trends

As discussed above, the development of colorimetric and optical sensors is highly attractive because of their real time operation and easy visualization. The review of the literature also revealed their great potential as intelligent package devices for the convenient, non-destructive and visual monitoring of meat spoilage, since pH value of samples is associated with their freshness. An ideal colorimetric indicator not only can exhibit excellent efficiency and accuracy, but also it has low cost and compact size to operate. In addition, reproducibility is equally important for manufactures. From all the reviewed studies of colorimetric sensors, the efficiency and accuracy were the essential problems, which were related to material adsorption ability for vapor amines, and also to their structures. Therefore, advances in improving efficiencies of indicators is largely credited to the novel properties of nanomaterials. CNCs and promising materials such as flakes of graphene (Z. Liu, et al., 2019), silicane and mesoporous carbon materials can alter the thickness, pore diameter, and structural order to further improve their sensitivity. The templating technique also has been used to create well-ordered nano-porosity to enhance the response. Apart from that, mixed conjugated polymers were used to intensify the color difference. Further studies must be conducted to set a correlation between the color change and the number of spoilage
microorganisms to accurately estimate the spoilage degree of food sample. Besides their durability, safety and cost also need to be considered. The impact of sensor media, and especially the synthetic dyes, on material recycling, composting, and disposal should also be under consideration. Combining different sensing concepts, rather than just one analyte-recognition, may be a well-practiced approach to improve sensor performance. Although the field of polymer-based colorimetric optical chemo-sensor is far from mature, the great collaboration with smart phones will provide impetus for development of freshness indicators that are integrated TTI indicators and enzyme biosensors. More advanced tools, such as electronic noses and electronic tongue, may hold enormous significance for food freshness industries. Thus, more efforts in development of advanced technologies are still needed.

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