Freshness indicators for real-time quality evaluation of packaged animal origin foods: A mini-review

N. R. Panjagari, R. K. Raman, K. Uma, R. Suwalka and E. Thomas

Abstract

Growing demand for safe and high-quality food has been driven by not only the rise in living standards but also consumers’ worry about food safety. The lack of real-time monitoring of perishable food freshness in the supply chain has led to the growing interest in improved food packaging systems. Among them, the freshness indicators have been proving to provide real-time quality status of the packaged food. They supplement or complement the printed “Best Before” or “Expiry Date” and give consumers an informed choice. Freshness indicators work on the principle of colorimetric changes with the changes in the headspace volatile components, an essential pre-requisite, of the packaged food product. An attempt has been made to review the recent developments in the freshness indicators of animal origin foods. Further, the chemistry behind the color changes in the freshness indicators due to continuous accumulation of spoilage off-flavors, and the criteria for the fabrication and the performance evaluation of freshness indicators have been presented briefly.

Key words: Colorimetric, Fish, Freshness indicator, Intelligent packaging, Meat, Milk

Highlights

- Freshness indicators are a subclass of smart packaging systems that conveys real-time quality information to consumers.
- The information displayed is by immediate visual changes, e.g., different color intensities or the diffusion of a dye along with the indicator geometry.
- Among all the animal origin foods, fish and meat have been extensively studied while very little work on the milk and milk products concerning freshness indicators.
- Headspace total volatile basic nitrogen (TVB), carbonyl compounds (aldehydes and ketones), carbon dioxide, and pH have been targeted to develop the freshness indicators.

Introduction

The quality of food deteriorates in the supply chain (mainly during distribution/storage) due to biological, chemical, biochemical, and physical processes. Delivering secure and safe food products within the maximum shelf life or “best before” or “expiry” dates of such food to consumers’ is one of the main goals of food packaging. With continuous innovations in packaging science, several techniques have been emerging to meet the consumer’s and other stakeholders’ requirements in the supply chain. Intelligent packaging systems comprise packaging systems that monitor the conditions of food to provide information about the quality throughout the supply chain. Intelligent packaging is briefly defined as the field of packaging science that facilitates decision making at stakeholders’ end by monitoring and communicating the conditions of the packaged food product (Yam and Lee, 2012). According to the European Commission Regulations, “materials and articles that are intended to monitor the condition of packaged food or the environment surrounding the food” are referred to as intelligent materials or articles.

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Intelligent packaging systems are classified into sensors, data carriers, and indicators (Table 1). All of them fall within the main category of “product quality and value-improving systems,” which are undoubtedly the widely used devices (Robertson and Queiroga, 2012). Indicators are a subclass of intelligent packaging tools whose characteristic feature is to convey the information through colorimetric changes. Despite the large varieties of indicators, all of them can be reasonably included within three categories: time-temperature indicators, gas indicators, and freshness indicators. From the consumers’ point of view, freshness indicators help them in deciding whether or not a packaged food is to be picked from the supermarket, thus giving them an informed choice. As of now, freshness indicators development has been focused on packaged aquatic food products (fish, shrimp), meat (chicken, beef, and carabeef), fresh horticultural produce (fruits/vegetables) but little on milk and dairy products. In this article, the concept of freshness indicators and their applications to animal-origin foods is presented.

### Freshness indicators
Among the intelligent or smart packaging systems, freshness indicators have been proving to provide real-time quality status of the packaged food. They supplement or complement the printed “best before” or “expiry date” and give consumers an informed choice. The genesis of the development of freshness indicators is based on the stakeholders’ need for the detection of spoilage of food through targeted metabolites associated with deterioration. Qualitative, as well as quantitative determination of volatile compounds, among others, gives an array of information regarding the changes associated with the food matrices. Freshness sensors or indicators are smart devices that are in direct contact with the internal headspace of a food package. They enable the monitoring of the quality of food products throughout storage and transportation and works on the principle of colorimetric changes with the changes in the headspace volatile components of the packaged food product. It directly provides the product quality information resulting from microbial growth or chemical changes within a food product (Siro, 2012; Yoshida et al., 2014). The reaction between the microbial growth metabolites and the integrated indicators within the package provides visual evidence regarding the microbial quality of the product (Kerry et al., 2006; Kuswandi et al., 2013a). Freshness indicators can also be used to provide an estimate of the remaining shelf life of perishable products. Understanding the metabolic reactions that are associated with the quality of a food product is essential for the development

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**Table 1. Classification of intelligent packaging systems**

| Sensors                        | Data carriers                      | Indicators                      |
|--------------------------------|------------------------------------|---------------------------------|
| Intelligent sensors            | Bar code                           | Time/temperature indicator      |
| Bio-sensors                    | Radio Frequency Identification (RFID)Tag | Oxygen indicator                |
| Gas sensors                    |                                    | Carbon dioxide indicator        |
| Fluorescence-based sensors     |                                    | Color indicator                 |
| Molecularly Imprinted polymer-based sensors |                                    | Pathogen indicator              |
| Quartz crystal microbalance    |                                    | Breakage indicator              |
|                                |                                    | Leak indicator                  |
|                                |                                    | Freshness indicator             |

Source: Modified from Vanderroost et al. (2014)
of a freshness indicator. Also, integration of freshness indicator with packaging material is one of the challenging tasks. Miller et al. (2006) state that the indicator generally needs to be placed inside the food package unless the packaging material is breathable or gas-permeable.

The working principle of a freshness indicator (direct/internal smart label) is illustrated in Fig. 1. It can be seen from the figure that the volatile quality markers such as total volatile basic nitrogen (TVB), carbon dioxide (CO₂), biogenic amines, ammonia, ethyl alcohol, and hydrogen sulfide interact with the freshness indicator label affixed from inside of the package especially primary package closure/lid. The interactions between the volatile markers and the freshness indicator label components are supposed to bring about responses through visual/colorimetric changes of the label. These changes could be easily seen from the specially provided opening in the secondary package. On the other hand, the indirect/external smart labels are placed over the secondary packages for monitoring the temperature, humidity, etc. They include time-temperature indicators, humidity indicators, and also radio frequency identification (RFID) tags for the supply chain management. In the case of indirect/external also, the changes could be visualized as they may contain thermo chromic inks as in the case of time-temperature indicators. Freshness indicators may be classified based on their sensitivity into (a) indicator sensitive to pH change (b) indicator sensitive to volatile nitrogen compounds (c) indicator sensitive to hydrogen sulfide (d) indicator sensitive to mixed microbial metabolite (e) indicator based on the release of certain nutrient release, and (f) indicator sensitive to other headspace volatile compounds.

**Criteria for the fabrication of freshness indicator for a food product**

The important criteria for the selection of a freshness indicator are to identify the key volatile headspace spoilage markers for a particular food product, formulation of smart ink components, selection of suitable polymer as supporting matrix, selection of a suitable technique for fabricating the freshness indicator containing the smart ink held by the selected polymer. Since a freshness indicator is unique for a particular food product or a group of food products, the volatile spoilage markers selection assumes importance. It can be seen from the literature that total volatile basic nitrogen (TVBN) has been chosen as spoilage markers for seafood (Kuswandi et al., 2012a), fish (Pacquit et al., 2006; Kuswandi et al., 2012b; Ezati et al., 2019), pork (Golasz et al., 2013), and beef (Kuswandi et al., 2015; Shukla et al.,...
2016) products while TVBN, hydrogen sulfide, and carbon dioxide for chicken (Kim et al., 2017; Zhai et al., 2019; Lu et al., 2020). In the case of egg yolk-based desserts, carbon dioxide was selected (Nopwinyuwong et al., 2010). However, for milk and milk products, aldehydes and ketones (Ziyaina et al., 2019; Goodarzi et al., 2020) have been considered as key spoilage markers for freshness indicator fabrication. Since the colorimetric changes are noticed due to the manifestations of microbial and chemical reactions in the food product over time, several dyes, both natural and synthetic have been tried. Among them redox dyes, curcumin, and anthocyanin extracted from different foods have been widely used (Kuswandi et al., 2012a; Golasz et al., 2013; Shukla et al., 2016; Zhai et al., 2019; Goodarzi et al., 2020). In general, color changes of pH dyes such as bromothymol blue, methyl red, bromocresol purple, bromocresol green, methyl orange, methyl yellow, and phenol red could be utilized to detect acidic/basic volatile compounds as these dyes exhibit irreversible changes in terms of structure and color upon dissolution. Among the polymer support matrices, thick papers, cellulose membrane, starch from different sources, methylcellulose, gums, hydrogels, and plastics have been utilized. Finally, for fabricating the freshness indicators, methods such as casting, absorption, or spinning method in which selected dyes or intelligent materials are mixed with a suitable solvent and fixed over the base or supporting material (Pacquit et al., 2006; Kuswandi et al., 2013b), casting, electro spinning, spin coating, ink-jet printing (Luo et al., 2021) have been adopted. Further, a list of key volatile compounds and their associated off-flavors of some animal-origin foods is presented in Table 2. Some of the studies on freshness indicators for different food products are listed in Table 3.

### Chemistry of interaction of freshness indicator dyes and headspace volatile compounds

Colour changes in the freshness indicators involve the chemical interaction between the targeted headspace volatiles and the intelligent materials. Based on these molecular interactions of dyes and targeted volatiles, colorimetric recognition of several molecules is described in the previous works. Fundamentally, molecular recognition involves the interactions between molecules i.e. bond formation, acid-base interactions, π-π molecular complexation, Vander-Waals interaction, and physical adsorption (Suslick et al., 2004). To monitor the warmed-off flavour defect in cooked chicken (resulting due to accumulation of pentanal, hexanal, and heptanal) a color sensor array was developed by Kim et al. (2016).

### Table 2. Major headspace volatile compounds of some animal origin foods and their corresponding off-flavors

| Product | Compound | Off-flavor | Reference |
|---------|----------|------------|-----------|
| Milk    | Free fatty acids (C₄₋C₁₀) | Rancid | Azzara and Campbell (1992) |
|         | Heptanal, octanal, nonanal, 2-octenal, 2-nonenal, hexanal, 1-octen-3-one, 2,4-decadienal, and acetaldehyde | Oxidized | Bassette (1976); Greig and Manning (1983) |
|         | 1-Octen-3-one and octanal | Cardboard | Hammond and Seals (1972) |
|         | 2-Alkenals (C7-C10) and alkanals | Tallow | Badings (1991) |
|         | 2- and 3-Methyl butanal | Malty | Morgan (1970a) |
|         | Ethyl butyrate and ethyl hexanoate | Fruity | Morgan (1970b) |

Table 2., Cont. ...
## Cont. Table 2. On-package freshness indicators of packaged animal origin foods

| Product                  | Compound                                                                 | Off-flavor                  | Reference                           |
|--------------------------|--------------------------------------------------------------------------|----------------------------|-------------------------------------|
| Skim milk                | Methanol and methyl mercaptan                                           | Oxidized                   | Allen and Parks (1975)              |
|                          | Hydrogen sulfide, methanethiol, and dimethyl sulfide                    | Cabbage                     | Jaddou et al. (1978)                |
| UHT milk                 | 2-Heptanone, 2-nonanone, octanal, nonanal, decanal, 2-methyl butanal, 2-methyl propanal | Stale                       | Zabbia et al. (2012)                |
| Sterilized concentrated milk | o-Amino acetophenone                                                    | Stale                       | Arnold et al. (1966)                |
| Yogurt                   | Acetic acid                                                             | Vinegary                    | Cheng (2010)                        |
| Butter                   | Acetaldehyde                                                            | Yogurt or green             | Lindsay and Rippe (1986)            |
|                          | Hexanal                                                                  | Oxidized                    | Christensen and Holmer (1996)       |
|                          | 1-Octane-3-one and 1,5(Z) octadien-3-one                                 | Metallic                    | Swoboda and Peers (1977)            |
| Butter oil               | 2-Nonenal                                                                | Cardboard                   | Widder and Grosch (1997)            |
| Ice cream                | Dimethyl disulfide and hexanal                                          | Putrid                      | Marsili (2003)                      |
| Sodium caseinate         | n-Alkanals, furan derivatives and O-amino acetophenone                  | Gluey                      | Ramshaw and Dunstone (1969)         |
| Cheddar cheese           | Ethyl butyrate and ethyl hexanoate                                      | Fruity                      | Morgan (1970b)                      |
| Smear coated cheese      | 2-Methoxy-3-isopropylpyrazines                                          | Potato                      | Dumont et al. (1983)                |
| Milk powder              | n-Alkanals, n-alkanones, furfural and benzaldehyde                      | Stale                       | Parks and Patton (1961); Schwambach and Peterson (2006) |
| Milk                     | pH changes                                                               | Acidic/basic odor           | Roy et al. (2021)                   |
| Shrimp                   | Ammonia                                                                  | Pungent (urine like)        | Ding et al. (2020)                  |
| Fish & Pork              | Ammonia                                                                  | Pungent (urine like)        | Ezati et al. (2021)                 |
| Tilapia                  | TVB-N                                                                    | Fishy                       | Yan et al. (2021)                   |
| Chicken                  | CO₂                                                                      | Sharp acidic odor           | Lu et al. (2020)                    |
| Shrimp                   | TVB-N, Ammonia                                                           | Fishy; Pungent (urine like) | Mohammadalinejehad et al. (2020)    |
| Fish                     | TVB-N                                                                    | Fishy                       | Wang et al. (2020)                  |
| Fish                     | pH & Ammonia                                                             | Fishy; Pungent (urine like) | Sani et al. (2021)                  |
Table 3. Freshness indicators developed for animal origin foods

| Product                  | Spoilage marker | Freshness indicator components | Supporting matrix | References                  |
|--------------------------|-----------------|--------------------------------|--------------------|-----------------------------|
| Beef                     | TVB-N           | Lichens extracts               | Litmus paper       | Kuswandi <i>et al.</i> (2015) |
| Beef                     | TVB-N           | Anthocyanin                    | Filter paper       | Shukla <i>et al.</i> (2016)  |
| Chicken                  | TVB-N           | Bromocresol purple             | Absorbent pad      | Kim <i>et al.</i> (2017)    |
| Chicken                  | CO₂             | Bromothymol blue/Methyl red    | Sugarcane bagasse (hydrogel) | Lu <i>et al.</i> (2020) |
| Chicken breast, Silver Carp | H₂S           | Silver nanoparticles           | Gellan gum         | Zhai <i>et al.</i> (2019)  |
| Fish                     | TVB-N           | Bromocresol green,             | Polyethylene       | Pacquit <i>et al.</i> (2006) |
| Fish                     | TVB-N           | Polyaniline                    | Polystyrene sheet  | Kuswandi <i>et al.</i> (2012b) |
| Fish                     | TVB-N           | Anthocyanin                    | Chitin nanofibre   | Wang <i>et al.</i> (2020)   |
| Fish                     | pH & Ammonia    | Red barberry anthocyanins      | Chitin nanofiber and methylcellulose | Sani <i>et al.</i> (2021) |
| Fish and Pork            | Ammonia         | Shikonin                       | Cellulose Paper    | Ezati <i>et al.</i> (2021)  |
| Golden drop              | CO₂             | Bromothymol blue and Methyl red| Methylcellulose    | Nopwinyuwong <i>et al.</i> (2010) |
| Milk                     | Volatile aldehydes and ketones | Schiff reagent                  | Silicon dioxide nanoparticles | Ziyaina <i>et al.</i> (2019) |
| Milk                     | pH change       | Black carrot anthocyanin       | Starch             | Goodarzi <i>et al.</i> (2020) |
| Milk                     | pH              | Shikonin& Propolis             | Gelatin & Carrageenan | Roy <i>et al.</i> (2021)     |
| Milk and Fish            | Volatile acids and bases | Red radish anthocyanin         | Gelatin, Gellan gum Cassava starch | Zhai <i>et al.</i> (2018) |
| Pork                     | TVB-N           | Anthocyanin                    | Cellulose paper    | Golasz <i>et al.</i> (2013)  |
| Rainbow Trout (Fish)     | TVB-N; Ammonia  | Alizarin                       | Cellulose Membrane | Ezati <i>et al.</i> (2019)  |
| Shrimp                   | TVB-N           | Curcumin                       | Modified cellulose & Polyvinyl Alcohol | Kuswandi <i>et al.</i> (2012a) |
| Shrimp                   | Ammonia         | Acidochromic dye               | Bacterial cellulose | Ding <i>et al.</i> (2020)   |
| Shrimp                   | TVB-N           | <i>Echium amoenum</i> (flower) | Polymeric chitosan (CH) | Mohammedalinejehad <i>et al.</i> (2020) |
| Tilapia                  | TVB-N           | Butterfly pudding flower       |                   | Yan <i>et al.</i> (2021)    |

Author reported that the developed sensor array was based on the cross-reactive mechanism between selected dyes mixed with 2-4 DNPH in presence of acid. The color changes in the array is due to the formation of hydrazide derivatives and water. Feng <i>et al.</i> (2010) developed a method for the detection of formaldehyde (gas) which works on the interaction between formaldehyde and primary amines leading to colorimetric changes. The
formation of hydrazine derivatives and H₂O which causes color changes. Kim et al. (2017) developed a freshness indicator to monitor the chicken freshness. The authors reported that the developed indicator relies on bromocresol purple dye immobilized with polyvinyl alcohol and a high moisture-absorbing material. The color of the indicator changes from yellow to blue and finally purple to indicate spoilage. Further, it was reported that sensor color change was in positive correlation with pH change with no migration of dye from the indicator onto the surface of chicken. Listyarini et al. (2018b) developed a freshness indicator film for monitoring meat spoilage that changes its colour from brownish violet to light yellowish-brown after its contact with ammonia gas in pH ranges of 7-11.

Integrating cellulose modified with acidochromic dye (ARC) into polyvinyl alcohol (PVA), Ding et al. (2020) produced an intelligent indicator with high strength and outstanding leakage resistance. At various pH levels, the produced indicator showed unique color changes. The dye was reported to be leakage-free in the acid solution and just 0.7 percent of dye was reported to be leaked in the alkaline solution, indicating that this pH film sensor has high leakage resistance. The PVA/ARC film sensor changed its color from yellow to brown within 24 h, demonstrating the sensor’s ability to monitor the shrimp quality in real-time. To monitor the warmed-off flavor defect in cooked chicken (due to the accumulation of pentanal, hexanal, and heptanal) a color sensor array was developed by Kim et al. (2016). The authors reported that the developed sensor array was based on the cross-reactive mechanism between selected dyes mixed with 2-4 DNPH in presence of acid. The array responds to the aldehydes based on the specific reaction between aldehydes and DNPH that leads to the formation of hydrazine derivatives and H₂O which causes color changes. Kim et al. (2017) developed a freshness indicator to monitor the chicken freshness. The authors reported that the developed indicator relies on bromocresol purple dye immobilized with polyvinyl alcohol and a high moisture-absorbing material. The color of the indicator changes from yellow to blue and finally purple to indicate spoilage. Further, it was reported that sensor color change was in positive correlation with pH change with no migration of dye from the indicator onto the surface of chicken. Listyarini et al. (2018b) developed a freshness indicator film for monitoring meat spoilage that changes its colour from brownish violet to light yellowish-brown after its contact with ammonia gas in pH ranges of 7-11.

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throughout a larger pH range of 2-11. Furthermore, both indicators were observed to have a high sensitivity and color reaction to ammonia, confirming a valid association between microbial growth, TVB-N production, pH changes, and indicator color changes. An innovative indicator was produced by Ezati et al. (2021) by adsorbing a pigment (naphthoquinone, shikonin) on cellulose paper to evaluate the freshness of fish and pork. Depending on the pH (2–12), the shikonin-adsorbed color indicator paper changed its color from red to blue and also changed the color sensitively to ammonia gas. It was reported that the color change of the indicator was closely related to the increase in the pH of fish and pork samples during storage at room temperature and the results showed a strong correlation between the color change of the indicator and the pH change of the sample.

**Freshness indicators of meat**

Carbon dioxide and TVBN compounds produced in meat products during storage have been widely considered as spoilage indicators for meat-based products. Yoshida et al. (2014) developed a colorimetric chitosan bio-based pH indicator for detecting n-butyrate, L-lactic acid, D-lactate, and acetic acid derived from microbial growth. Carbon dioxide (CO$_2$) indicators consisting of aqueous solutions of chitosan or whey protein isolate were used to detect the changes in transparency of the indicator due to the pH-dependent whey (Jung et al., 2012). Rukchon et al. (2014) developed a colorimetric freshness indicator for skinless chicken breast spoilage and reported that the indicator responded to CO$_2$ and changes its color. The indicator response correlated well with microbial growth and the number of volatile compounds generated thus enabling real-time monitoring of food spoilage. Shukla et al. (2015) developed a colorimetric freshness indicator to monitor the freshness of buffalo meat during refrigerated storage. The fabricated indicator changed its color from yellow to blue as the meat quality deteriorated in terms of accumulated TVBN in the package headspace. Further, based on the changes in the color, the authors developed a color scale to measure the degree of freshness. Chen et al. (2019) developed a freshness indicator label for assessing meat freshness. The indicator label was made by mixing the suspension of methylcellulose, polyethylene glycol-6000, and pH-sensitive dyes either singly or in combination. Indicator labels change their color from blue to yellow in response to spoilage. The color response was correlated with changes in pH, TVB-N content, and aerobic plate counts of pork. Further, using statistical models color changes of the label were successfully predicted for TVB-N contents and aerobic plate counts of pork. A freshness indicator based on high moisture-binding materials was developed by Kim et al. (2017) to monitor the chicken freshness. The color of the indicator containing bromocresol purple changes from yellow to blue and finally purple to indicate spoilage which positively correlated with pH change. Further, it was reported there was no migration of dye from the indicator onto the surface of chicken was documented. Lee et al. (2019) developed a freshness indicator to monitor chicken breast spoilage. Color changes in the indicator were monitored with the naked eye as well as smartphone and processed digitally. Concentration-based optimal color changes in indicators were reported in simulated conditions using 8 different concentrations of trimethylamine.

**Freshness indicators of milk products**

Studies on the development of freshness indicators on milk and milk products are negligible compared to fish and meat. Kulchan et al. (2016) developed a novel mixed pH dye-based colorimetric indicator for monitoring rancidity reaction in infant milk powder formula. The indicator was based on pH-sensitive dyes either singly or in combination which was supported on methylcellulose and hydroxypropyl methylcellulose as fabricating material. Developed indicator monitors the
rancidity by sensing hexanal and acetic acid as spoilage metabolite and responds through a color change from bright green for fresh to orange as an indication of warning. Color changes of the indicator were in agreement with the chemical quality indices like peroxide value and free fatty acids and sensory score. Among the dairy products manufactured in Indian sub-continent, traditional dairy products have social, cultural and economic importance. Although majority of them are produced at small scale with the continuous research and development (R&D) efforts from both academia and industry, they are now being produced at industrial scale with improved processes and packaging systems. Improved packaging systems such as modifying the package internal atmosphere (air) with near zero oxygen content and flushing it with inert nitrogen gas (modified atmosphere packaging); evacuating the entire ambient air from the package (vacuum packaging); and utilization of oxygen scavengers for selective removal of oxygen from the headspace (active packaging) are some of the popular ones. In addition, improved package design (presentation of products and label graphics) has been another area where the manufacturers have been focusing to meet the consumers’ demands. However, the use of novel packaging systems such as freshness indicators which can give a non-destructive and real-time quality status details to the consumers and helps in having an informed choice at the supermarkets’ shelves, has not yet been adopted mainly due to the lack of available technologies. In view of the importance and potential export market for traditional Indian dairy products, studies have been initiated at ICAR-NDRI in the authors’ laboratory. The freshness indicators for *Khoa, Sandesh*, and *Dahi* have been developed and filed for protection as a patent. Hence, we may expect these indicators in the market in the near future to address the food safety concerns.

**Freshness indicators of other animal origin foods**

*Nopwinyuwong et al.* (2010) developed a freshness indicator to monitor the quality of Golden drop, an egg yolk-based intermediate moisture Thai dessert which could serve as a “chemical barcode” for real-time monitoring of spoilage. The indicator contained mixed pH-sensitive dyes, bromothymol blue, and methyl red that respond to carbon dioxide (CO₂) as a spoilage metabolite through visible color change, which correlated well with CO₂ levels. *Listyarini et al.* (2018a) assessed the indicator response against the shrimp volatiles during storage (2 h, 17 h and 24 h) by placing the indicator label in a clear glass vial and observed color change in the indicator label due to decay of spoilage.

**Performance evaluation of freshness indicators**

Freshness indicators react selectively with the volatile compounds in the headspace of the package and respond through a visual color change. By reviewing the literature, it was observed that the sensitivity of the freshness indicators is mainly evaluated by three different measures namely color response (a) to different pH (b) to a target analyte in a liquid phase (c) to a target analyte in a vapor phase. In the colorimetric response of the indicator labels to different pH, an indicator is evaluated due to changes in the acidity or basicity of the package environment. *Zhai et al.* (2019) evaluated the response of a freshness indicator of milk and fish based on the changes in acidity. The indicator film changes its color from orange-red to yellow in the pH range of 2-12. *Listyarini et al.* (2018 b) developed a freshness indicator film for monitoring meat spoilage that changes its color due to changes in the basicity of the package environment from brownish violet to light yellowish-brown after contact with ammonia gas in pH ranges of 7-11. A universal pH indicator based on halochromic dyes (phenol red, methyl red, bromothymol blue, phenolphthalein, and bromocresol green) was developed to assess the pH variations. The developed indicator was capable of detecting pH values from 1-10 indicating a typical color at each pH (*Agarwal et al.*, 2012).
colorimetric response to a target analyte in the liquid phase method, a definite amount of target analyte is directly dropped on the surface of the indicator label and the color changes in the indicator label are recorded and analyzed. Listyarini et al. (2018a) developed a freshness indicator film for monitoring shrimp spoilage and its response to liquid ammonia (0.8M) was evaluated by placing the film (disc-shaped) on the surface of pH buffer solutions (pH 7-11) in a petri dish, and the color changes were recorded and analyzed. The response of freshness indicators is also assessed by exposing the indicators to a known concentration of gaseous analyte at a particular temperature by placing them in a gas-tight clear glass vial. This method is also suitable to measure the concentration-dependent color changes. Nopwinyuwong et al. (2010) assessed the concentration-dependent color changes in freshness indicator intended to measure the freshness of golden drop (a Thai dessert) and reported a brilliant color transition in the indicator label due to exposure to varying concentrations of CO$_2$.

Commercially available freshness indicators

According to Kuswandi (2017), freshness sensors are classified into three types based on the number of sensors they contain: single, dual, and multiple. Single sensors are those freshness sensors in which only one sensor is used for sensing the target analyte and communicating it to the observer. While the dual freshness sensors/indicators are those in which two sensors are employed to refer to each other in detecting the target analyte and also communicating it to the consumer about the quality of food. The third one i.e. multiple freshness sensors/indicator consist of more than two sensors which ultimately make an array to make a pattern that can be utilized as a tool for sensing the analyte and communication for the consumers. However, currently, single-type freshness sensors are common as compared to dual and multiple type sensors are commercially popular. Commercial applications of freshness indicators include SensorQ™ by FQSI Inc (United States of America), which senses spoilage in fresh meat and poultry products and Toxinguard® by Toxin Alert Inc (Canada) to monitor Pseudomonas sp. growth (Kerry and Butler, 2008). COX Technologies, USA launched Fresh Tag® colorimetric indicator labels that react with volatile amines produced during the storage of fish and seafood products (Hogan and Kerry, 2008). The Vanprob Company developed food fresh indicators (time-temperature based. Insignia Technologies, produced NOVAS freshness indicator, which is easily incorporated into film lid (https://www.insigniatechnologies.com).

**Freshness indicator development: Challenges**

Freshness Indicators offers more direct information than other contemporary indicators such as TTIs and leak indicators. Despite this apparent advantage freshness indicators have generally been far less successful than TTIs in gaining a market foothold (Smolander, 2008). Other loopholes of freshness indicators are color changes due to contamination can result in false-positive results (products may not have any significant sensory or quality deterioration). The presence of certain target individual metabolites is not necessarily an indication of poor quality and chances are there for a generation of false-negative or false-positive results, which may dissuade consumers regarding product quality (Hogan and Kerry, 2008). To eradicate all these hindrances, the developed indicator must be characterized with the targeted metabolite or analyte in simulated conditions, and developed color, as well as their intensity, should be cross-checked with real-time product spoilage during storage.

**Conclusion**

Delivering safe food products within the claimed shelf life or “best before” or “expiry” dates of such food is one of the main goals of food packaging. Several techniques have been emerging to meet the consumer’s and other stakeholders’ food safety and assurance in the supply chain. On-package food freshness indicators have been a result of continuous
developments in packaging science that meet that demand. Several volatile compounds especially TVBN, carbon dioxide, ammonia, and carbonyl compounds (ketones and aldehydes) have been considered for the fabrication of freshness indicators for perishable animal origin foods. Further, a wide range of polymer support matrices is compatible with freshness indicator development. Since already a good number of freshness indicators are available in the global market, great scope exists for the Indian fresh and processed traditional animal origin foods.

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