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Development of Colorimetric On-package Indicator for Monitoring of Chicken Meat Freshness during Refrigerated Storage (4±1°C)

Arvind Soni*, Sanjod Kumar Mendiratta, Suman Talukder, Rajiv Ranjan Kumar, Sagar Chand and Rohit Kumar Jaiswal

Division of Livestock Products Technology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, INDIA

**Corresponding author: A Soni; Email: lugaks3311@gmail.com*

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ABSTRACT

Real-time quality monitoring of packaged meat is the key of today's commercialized meat sector to ensure its safety in supply chain. Designing of user friendly dye based cost effective indicator can fulfil all the present requirements. Bromocresol Purple (BCP) is chemo-responsive dye, has been evaluated to develop colorimetric indicator. The indicator (0.1% of dye in 50% ethanol) was fabricated by immobilized BCP dye on indicator carrier (2% w/v agarose gel) to make strips of specific dimension (2 cm^2). Indicator strip was attached inside fresh meat packets, such a way that it could be visible from outside of the packet during storage at 4±1ºC. Dye was immobilized in agarose gel to make the strips as an indicator, which worked during increase in concentration of total volatile basic nitrogen (TVBN) and ammonia produced gradually in the chicken meat package headspace. The colour changes in indicator were observed from light yellow to purple, with concurrent spoilage of chicken meat. The changes in colour of indicator strip were compared with chicken meat quality parameters upto day 7 with intervals of 2 days during storage at 4±1°C. pH, Thiobarbituric Acid Reacting substance (TBARS), TVBN, Ammonia level, Free amino acid (FAA) and microbial counts increased ($P<0.05$) significantly during storage. Results have indicated that indicator colour response correlated well with the change in physico-chemical and microbial parameters of chicken meat, making the indicator enable to real-time monitoring of chicken meat during the storage at 4±1°C.

Keywords: Real-time monitoring, Indicator, TVBN, Chicken Meat, Refrigerated Storage

Since time immemorial food is packaged for storage, preservation, protection and transportation to deliver good quality edibles to consumers. The package is still used today for protecting the food items from adverse conditions of atmosphere and for better maintenance of quality and safety in processing chain. Now a day's, the demand of packaged food has increased due to urbanization and fast face of life which focuses in terms of user convenience (Butler, 2004). Fresh meat is nutrient rich medium (Nychas *et al.,* 2008) for growth of microbes, which spoiled early due to improper packaging and storage. The increased incidences of meat borne diseases due to intake of unsafe packaged meat is not uncommon, therefore, it is mandatory to check the wholesomeness, quality and safety of fresh meat throughout the supply chain.

India has huge livestock potential but the meat sector is still unorganized, lacking scientific and technical support. Among the various meat, chicken meat consumption increased tremendously in India due to its association with a more healthy diet, relative inexpensive protein source, and because it preferred by all community. Total annual estimated meat production in India during 2016-17 was 7.0 million tones, in which poultry meat shared about 46%. Poultry industry is fast growing sector which contributes 1.0 percent India's GDP and 11.70 percent of the GDP from the livestock sector with production of approx 3.22 million tones of meat (DAHD & F, 2016-17). The country has exported 0.6 million tonnes of poultry products to the world worth ₹ 768.72 crores in 2015-16 (APEDA, 2015-16).

Indian food industry faces struggle in the meat business due to inefficient cold storage services, scientific packaging, preservation and efficient transport system which leads us to the state, where the meat spoilage rate is highest in the world. Food travels to the Indian consumers through a sluggish and inefficient cold chain. Deterioration of meat qualities is mainly due to improper cold chain facilities, which creates huge economic loss to producers and hits the trust of consumers. Meat and meat based products are considered as highly perishable commodities; most critical factor is to manage the temperature throughout the supply chain. Fluctuation in temperature at any point during transport and consequent storage, leads to decreased acceptance of the products or complete rejection due to the deteriorative changes. Therefore, there is a need for an efficient system which determines the meat quality in real-time scenario during supply chain. Recently, demand for real-time quality monitoring of meat is increased by consumers and manufacturers. To accomplish this task, intelligent packaging may be emerged as boon that facilitates the sensing of physico-chemical changes occurring in meat through irreversible visible colour response of the indicator.

Intelligent packaging system includes freshness/quality indicator, which are cost effective, quick and non-destructive system to determine the quality of perishable products as well as to evaluate technical procedures on the basis of colour changes of indicator (Talukder *et al.,* 2017). Indicator that are able to monitor volatile organic compounds and gas molecules such as H_2, CO_2, H_2S, NH_3, CH_4 and total volatile basic nitrogen (TVBN) related to food spoilage are particularly required (Vanderroost *et al*., 2014). The indicator colour response found to correlate with quality changes in food items especially bacterial growth patterns in most of the studies, thus enabling the ''real-time'' monitoring of spoilage. These colorimetric indicator offers the potential of developing dynamic ''best-before'' dates that may lead to important and exciting improvements in the quality assurance sector. Different type of quality indicators have been developed for application in intelligent packaging utilizing different indicator solutions and carrier materials. Shukla *et al.* (2016) used rose and cabbage based natural dye and Talukder *et al.* (2017) used ripen black mulberry (*Morus nigra*) fruit extracts to develop filter paper based quality indicator. Natural dye based indicators are more safe and cheap but there are huge variation in the degree and visibility of colour change, which instigate the researcher towards use of chemical dye as a better source, which are more chromomeric in nature. A colorimetric Bromocresol Purple (BCP) dye-based pH-responsive indicator was developed to monitor the quality of chicken breast meat by direct surface contact (Kim *et al.,* 2017). There is chance to release the chemicals from chemical dye based indicators which may detoriate the quality and safety of meat. In the present study efforts was made to develop safest form of indicator with minimum chance of leaching to the meat system without hampering its colour visibility. So, with considering above points, a chemical dye based quality indicator was developed to monitor the freshness of chicken meat at refrigeration temperature.

MATERIALS AND METHODS

All the analytical grade chemicals were purchased from standard firms (Sigma-Aldrich, Merck, SRL, Hi-Media, *etc*.) and the indicator carriers and accessory materials like agarose, filter papers, etc. were purchased from standard firms (Merck, SRL). PET (Polyethylene terephthalate) boxes and LDPE (Low Density Polyethylene) will be used as packaging materials and were purchased from local market of Bareilly. Fresh chicken meat sample (White Leghorn) of normal pH (5.6-5.7) required for the experiments was procured from Division of Post Harvest Technology, ICAR-CARI, Izatnagar and from experimental abattoir of Livestock Products Technology Division, ICAR-IVRI, Izatnagar, Bareilly. Study was carried out in the Division of Livestock Products Technology, ICAR-Indian Veterinary Research Institute, Bareilly.

Development of Bromocresol Purple dye base freshness indicator

Indicator solution

A solution of the powered dye (0.1% w/v) was prepared by using 50% ethyl alcohol (v/v) as solvent. The chemical dye was selected on the basis of their ability to change colour at different pH, sensitivity to volatile bases, stability at ambient temperature and stability with carrier.

Indicator carrier

With the selected chemo-responsive dye, different carrier forms of indicators were prepared and trials were conducted to determine their efficacy to visible colour change in chicken meat PET boxes with attached indicator forms. A agarose gel strip type indicator, (2% (w/v) agarose solution in distilled water) was made with continuous stirring and heating at 90°C temperature and 550 rpm rotation by using hot plate with magnetic stirrer (Model 11603, Merck Specialties Pvt. Ltd.) for 30 min. After this, solution was cooled to 45°C and added 15% (v/v) dye solutions with proper mixing. Mixture was then poured into a petri-dish of diameter 100 mm and allowed to solidify at room temperature. A 2cm×2cm size of strip was cut and packed in LDPE film to make a sachet for avoid accidental leak of dyes, stored in dark condition at room temperature in laminated pouches and should be utilized within 30 days.

Evaluation of response of colour change of developed indicator

The developed agarose gel strip type indicator was tested for their color changing response with chicken meat at refrigeration (4±1°C). 100g of chicken meat was taken in PET box and indicator was attached to the inner side of the lid with the help of transparent sticky tape. Close the lid air tightly keeping the indicator in an atmosphere inside the PET box. Samples were kept at refrigeration (4±1°C) upto the point where visible sign of spoilage observed, and different quality and microbial parameters were evaluated with concurrent changes in colour of indicator at alternate days *i.e.* day 1,3,5,7. Changes in the color of the indicator were recorded by digital camera (SX160 IS, Canon, India).

Evaluation of physico-chemical and microbiological changes in stored meat

Changes in physico-chemical and microbiological parameters related to meat quality were observed during refrigerated storage to correlate between gradual changes in the indicator colour and meat quality. Various physicochemical parameters *viz.* pH (Trout *et al.,* 1992), TBARS (Thiobarbituric Acid Reacting substance) (Tarladgis *et al.,* 1960), TVBN (Pearson, 1968), ammonia level (Sastry *et al.,* 1999), free amino acids (Rosen, 1957), hunter colour values (Mini Scan EZ Colour Meter, Illuminant D65, 2.5 cm diameter aperture, 10° standard observer, Model No. 4500 L,USA) and microbiological parameters *viz.* total plate count, psychrophilic count and *Pseudomonas* count (APHA, 2001) were evaluated by respective standard methods.

Statistical analysis

Experiments were repeated a minimum of three times and the data generated were compiled and analysed using SPSS (version 20.0 for windows; SPSS, Chicago, III., U.S.A.). The data were subjected to one way ANOVA, Mean±S.E using SPSS software package developed as per the procedure of Snedecor and Cochran, 1995 and means were compared by using Dunkan's multiple range test (Dunkan, 1955).

RESULTS AND DISCUSSION

Colour response of indicator with chicken meat under refrigerated storage (4±1°C)

Colour response of Bromocresol Purple based freshness indicator was observed for chicken meat at 4±1°C. Indicator changed its colour from initial light yellow to final purple on 7th day. During storage different volatile basic gases were generated inside the packets, which might have caused the color changes of indicator from initial light yellow to final purple at the end of 7th days of storage (Fig. 1).

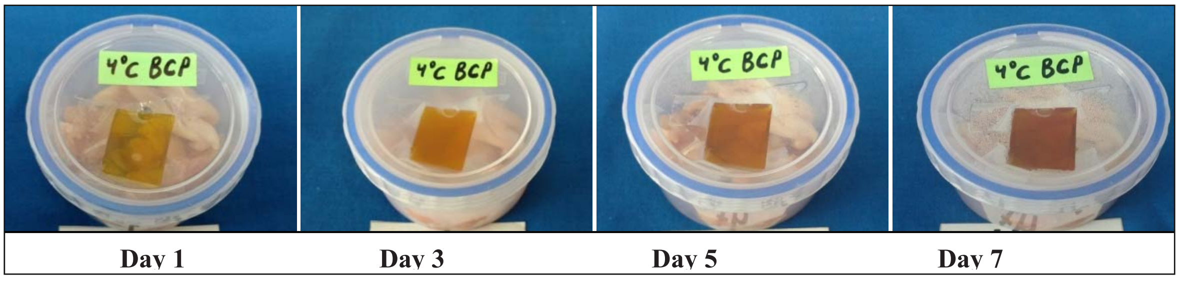

Fig. 1: Color changes of indicator during storage of chicken meat packets at refrigeration temperature (4±1ºC)

Correlation of colour response of indicator with changes in physico-chemical quality of chicken meat

Correlation of the colour response of the developed indicator with the quality changes of fresh chicken meat was studied during refrigerated storage. Chicken meat samples were packed in PET boxes and stored along with indicators. At a particular interval of time. Samples were collected from packaged chicken meat and studied for different quality parameters. The colour response of the quality indicator was compared simultaneously with the meat quality parameters in order to relate colour response with the acceptability of stored samples. pH, TBARS value, TVBN, Ammonia level, FAA value of chicken meat increased significantly (P<0.05) with parallel changes in colour of the indicator during refrigerated storage (Table 1). Increase in pH level was usually related to the bacterial count of meat that resulted in the production of ammonia, amines and other alkaline substances and accumulation of basic metabolites resulting from protein degradation by microbes (Nychas *et al.,* 1998). A similar increasing trend of pH was reported in of chicken breast (Kim *et al.*, 2017) and in chicken cut (Kuswandi *et al.,* 2014) at refrigerated (4±1°C) storage.

TBARS value increased significantly (P<0.05) throughout the storage period from 0.33 on day 1 to 1.52 mg malonaldehyde/Kg on day 7. Increased value was might be accounted to lipid oxidation and volatile metabolites (Brewer *et al*., 1992). Result corroborates well with the findings of Park *et al.* (2013) and Ali *et al.* (2007) in chicken breast meat during storage at refrigeration temperature.

The TVBN content increased (Fig. 2) significantly (P<0.05) with the change in colour of the indicator and reached beyond the recommended limit of 20 mg/100 g (Byun *et al.,* 2003) on 7th day of storage. Increase in TVBN and ammonia concentrations during the storage was might be due to breakdown of protein and deamination of amino acids.

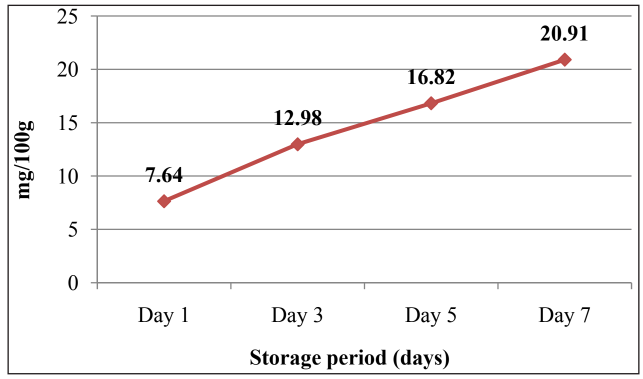

Fig. 2: Changes of TVBN content of chicken meat stored at refrigeration (4±1ºC)

The psychrophilic bacteria mostly *Pseudomonas* growing on meat deaminate amino acids and produce volatile bases (Barnes and Ingram, 1955). The obtained results are comparable to the results obtained in the studies by Nopwinyuwong *et al*. (2010) and Rukchon *et al.* (2014). The result also agreed with the findings of Kozacinski *et al*. (2012) in pre-packed cut chicken meat at refrigerated storage and they found, that chicken meat spoiled at ammonia level of 9 mg/g. FAA level of chicken meat was

Table 1: Physico-chemical parameters of chicken meat during storage at refrigeration (4±1)°C temperature (Mean±S.E.)*

Parameters	Storage period (days)			
	Day 1	Day 3	Day 5	Day 7
pH	5.78±0.03^{c}	5.89±0.02^{c}	6.16±0.04^{b}	6.34±0.02^{a}
TBARS value (mg malonaldehyde/Kg)	0.33±0.03^{d}	0.64±0.04^{c}	1.10±0.04^{b}	1.52±0.06^{a}
TVBN (mg/100g)	7.64±0.25^{d}	12.98±0.18^{c}	16.82±0.21^{b}	20.91±0.22^{a}
Ammonia (mg/100g)	25.9±0.54^{d}	35.23±0.52^{c}	38.78±0.4^{b}	56.0±1.49^{a}
Free amino acid (mg/100g)	61.53±2.87^{d}	80.18±2.28^{c}	100.57±2.66^{b}	126.93±2.68^{a}
Lightness (*L**)	54.34±0.70^{bc}	53.71±0.43^{c}	56.31±0.46^{ab}	57.11±0.46^{a}
Redness (*a**)	3.19±0.23^{ab}	3.81±0.42^{a}	2.48±0.16^{b}	1.26±0.11^{c}
Yellowness (*b**)	7.31±0.44^{c}	10.64±0.48^{b}	12.87±0.22^{a}	14.03±0.21^{a}

n =6, *Mean±S.E. with different superscripts indicate significant (P<0.05) difference.

found 61.53 mg/100g on day 1 which increased to 126.93 mg/100g on day 7 during storage. It has been shown that sum of free amino acids along with water soluble protein content increased during storage and this corresponded well with colony counts, particularly with meat having high glucose concentration (Nychas and Tassou, 1997). Similarly, an increase in concentration of FAA was observed during storage in turkey breast (Przysiezna, 2005).

Lightness (*L**) and yellowness (*b**) values of chicken meat increased significantly (P<0.05), while redness (a*) value decreased significantly (P<0.05) with collateral change in colour of indicator (Table 1). Colour values of poultry meat for *L**, *a** and *b** coordinates were varies between 38.2-41.6, 8.27-8.97 and 7.6-11.7 (Zhao *et al*., 2012). For chicken meat, decrease in redness (*a**) was also recorded by Yang and Chen, (1993) in ground chicken meat at refrigerated storage. Ali *et al.* (2007) and Talukder *et al.* (2017) also observed decrease in redness (*a**) value refrigeration in chicken meat. The decrease in redness value was probably due to formation of metmyoglobin which imparted brownish discolouration to meat. Increasing trends of yellowness (*b**) value has been showed by Zhang *et al.* (2016) for raw chicken meat and by Rababah *et al.* (2006) for cooked chicken breast during refrigerated storage. The chicken breast stored at 4°C showed decrease in lightness value *i.e.* surface colour of the chicken breasts changes from pink to red violet and finally dark purple or bluish-red (Kim *et al.,* 2017).

Correlation of colour response of indicator with changes in microbial parameters of chicken meat

Significant increase (P<0.05) in Total Plate Count (TPC), psychrophilic count and *Pseudomonas* count was observed in chicken meat during storage with a collateral changes in the colour of indicator (Fig. 3). At refrigeration temperature, higher TPC could be due to proliferation of already existing micro flora. Microbial spoilage is of great concern for fresh poultry meat as it has a limited storage life under refrigerated conditions (Chouliara *et al.,* 2008). The limit of acceptability for human consumption is 6-7 $\log_{10}$ cfu/g microorganisms for fresh poultry (Rukchon *et al.,* 2014). The present result of TPC corroborates well with finding of Park *et al*. (2013) in chicken breast meat stored at 5°C temperature. An increasing trend in TPC of aerobically packaged poultry meat from 4.60-6.38 $\log_{10}$ cfu/g on 0 to 4 days at 4ºC was reported by Zhang *et al.* (2012). Senter *et al.* (2000) also reported increasing trend in TPC value from 4.08 on 0 day to 6.57 $\log_{10}$ cfu/g on day 5 in skinless chicken breast at 4°C. Refrigeration temperature (4±1ºC) does not allow growth of majority of the microbes, but some of the psychrophiles can grow and may cause spoilage which could be detected by change in colour of indicator. Psychrophilic count was not detected on 1st day, while it increased significantly (P<0.05) to 4.73 $\log_{10}$ cfu/g on 7th day of storage (Fig 3). A similar increasing trend was reported in broiler cuts stored at 5±1° C temperature (Smolander *et al.,* 2002). Byrd *et al.* (2011) reported increase in psychrophilic count from 2.58 on 0 day to 7.05 $\log_{10}$ cfu/g on 14 day at 2º C in chicken meat. Huang *et al*. (2011) also reported increase in psychrophilic counts for boneless and skinless broiler chicken meat at 4ºC from 4.27 $\log_{10}$ cfu/g on 0 day to 6.29 $\log_{10}$ cfu/g on 9 day.

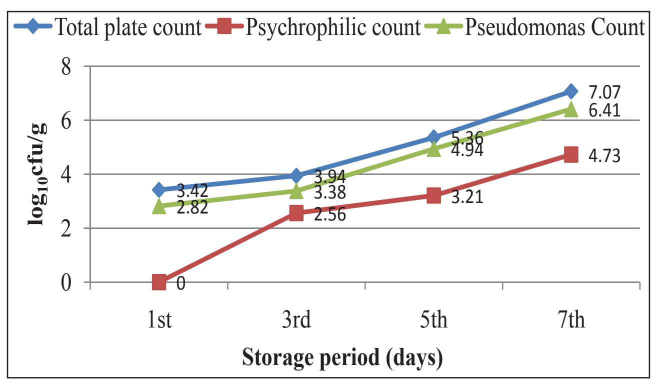

Fig. 3: Changes of microbiological count of chicken meat stored at refrigeration (4±1ºC)

As *pseudomonas* is a psychrophilic bacterium and refrigeration temperature (4±1ºC) favours its growth. *Pseudomonads,* the dominant microbial species in aerobically packaged poultry, are considered to be the prevalent cause of spoilage of poultry meat and meat products (Patsias *et al*., 2008). Obtained result indicated significant (P<0.05) increase in *Pseudononas* count (Fig. 3) which was also reported by Balamatsia *et al.* (2007) in chicken meat and Rukchon *et al.* (2014) in skinless chicken breast during aerobic storage at 4±1ºC. Similar increasing result was also reported by Hinton *et al.* (2004) for packaged broiler meat.

CONCLUSION

During refrigerated storage of chicken meat, BCP dye based indicator changed its color from light yellow to purple due to chemical reaction occurring between dye and volatile bases generated in PET boxes. Finding of present study indicated that developed indicator represents a simple and cost effective visual aid to detect quality degradation of flesh foods.

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