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Application of urease based enzymatic time temperature indicator (TTI) as thermal abuse marker for frozen chicken meat quality

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Abstract
Urease based TTI was prepared using urea as substrate and enzyme urease to monitor thermal abuse history of frozen chicken meat during supply chain. TTI showed an irreversible distinct colour change from initial yellow to intermediate light red to final pink colour during temperature abuse conditions. At different temperature abuse conditions *viz.* 5±1°C, 15±1°C, 25±1°C and 35±1°C, quantitative changes in various parameters of chicken meat were evaluated and correlated with distinct colour alterations of TTI. The results showed insignificant correlation of meat quality parameters with colour changes in TTI at 5±1°C, 15±1°C, 25±1°C and 35±1°C. So, changes in quality characteristics of frozen chicken meat are not precisely indicated by TTI after exposure to different temperature abuse conditions. Therefore, urease based enzymatic TTI cannot be utilized for quality marking of frozen chicken meat at different temperature abuse conditions.

Keywords: Time temperature indicator, urease, chicken meat, temperature abuse

1. Introduction

India is imparted with massive livestock resources with a population of 529 million and 729 million poultry; growing at the rate of 6% per annum. There is increase in the contribution of livestock to Gross Domestic Product (GDP) of country which accounts for 17.5% of total agricultural sector and 4.5% of GDP (DADF, 2014) [1]. Meat production is estimated at 7.7 million tonnes/year and per capita availability of 2.96 kg/year, with major contribution from poultry meat *i.e.,* nearly about 49.64 % of total meat production in India (BAH & FS, 2018) [2]. Poultry meat has gained importance due to its cost competitiveness, nutritional quality, universal availability and freedom from religious taboos. The attributes of poultry meat *viz.* higher protein, low fat and cholesterol content are in concordant with modern consumer's demand profile. As far as perishable commodities like meat and meat based foods are concerned, most crucial factor is the control of temperature throughout supply chain. Due to the heterogeneous temperature during supply chain, proper monitoring of the temperature along the cold chain remains a significant challenge resulting in quality deterioration (Pelletier *et al.,* 2011; Raab *et al.,* 2008) [3, 4]. Therefore, an efficient system that can monitor the changes in quality during supply chain or a system that can detect exposure of meat to any detrimental factor is needed to check the down grading of meat quality in supply chain. Intelligent/smart packaging systems are a critical solution for monitoring quality of meat and meat products, to predict its remaining shelf life during storage period because of its far reaching potentiality, from food safety to postal delivery tracking. Intelligent packaging systems attached as labels, incorporated into, or printed onto a food packaging material offer enhanced possibilities to monitor product quality, trace the critical points and give more detailed information throughout the supply chain (Han *et al.,* 2005) [5]. This system includes various indicators *viz.* quality, integrity, freshness and time-temperature (TTI) indicators which ensures food safety to consumer. Among TTIs, enzymes based TTI have many benefits over other types due to unwavering performance, low production cost and easy to apply (Jaiswal *et al.,* 2018) [6]. With this background, urease based enzymatic TTI was applied to frozen chicken meat for establishment of correlation with various meat qualities attributes at different temperature abuse conditions.

2. Materials and Methods
2.1 Preparation of urease based enzymatic TTI
The urease based enzymatic TTI using the reaction between enzyme urease and substrate urea was prepared after suitable modification in the procedure standardized by Singh (2018) [7] with subsequent observation for intermediate and final irreversible colour change pattern at 5±1°C, 15±1°C, 25±1°C and 35±1°C.

Sample preparation

For dynamic temperature abuse study, 150g of chicken meat was packed in LDPE bags (100 gauge) and TTI was attached to it and stored in deep freezer (-18±2°C) for 24h before exposing to temperature abuse conditions. The conditions were simulated in laboratory by using incubator and refrigerator. Temperature conditions were monitored by using probe thermometer (Digi-thermo, WT-2, China) and meat samples were exposed to temperature abuse conditions of 5±1°C, 15±1°C, 25±1°C and 35±1°C. At intermediate and final irreversible colour alterations, meat quality parameters were measured and changes in various meat quality attributes were correlated with initial, intermediate and final colour changes.

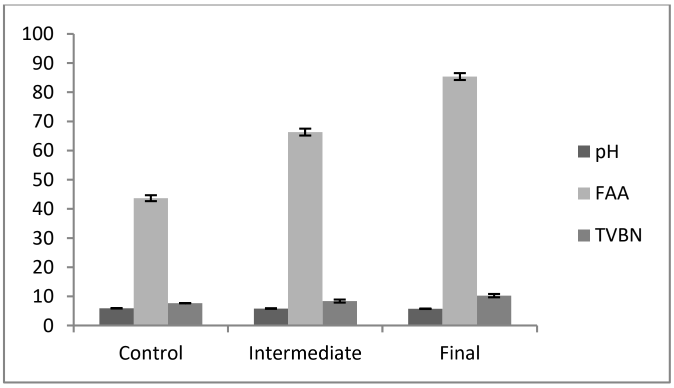
2.2 Determination of meat quality parameters with colour changes in lipase based enzymatic TTI
Various physico-chemical parameters like pH (Trout *et al*., 1992) [8], free amino acids (Rosen, 1957) [9], total volatile basic nitrogen (Pearson, 1968) [10], aerobic plate count and psychrophilic count (APHA, 2001) [11] were estimated with concurrent change in the colour of TTI at different temperature abuse conditions. Besides this, instrumental colour analysis *i.e.*, redness (a value) and yellowness (b value) using Lovibond Tintometer (Froehlich *et al.*, 1983) [12] were also performed.

2.3 Statistical Analysis
Each experiment was repeated three times and the data generated for different meat quality parameters were compiled and analysed using one way analysis of variance with SPSS (Version 20.0 for Windows; SPSS, Chicago. 111., U.S.A.).

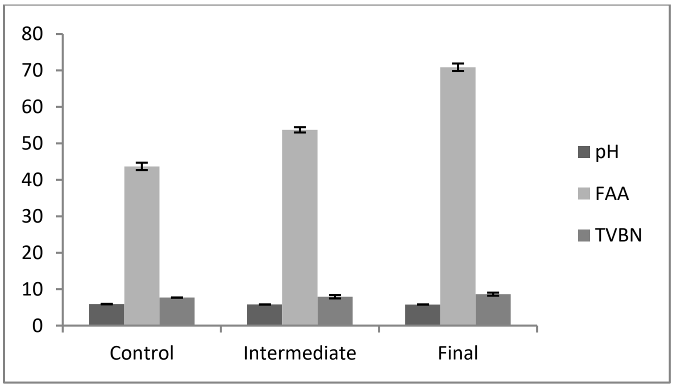
3. Result and Discussion
3.1 Colour changing pattern of urease based enzymatic TTI
The TTI colour response at different exposure temperature was compared with a control TTI kept at -18±2°C. The time taken from initial yellow to intermediate light red and final pink colour irreversible response by TTI at different temperature are 15h and 24h (5±1°C), 9h and 12h (15±1°C), 3.5h and 5h (25±1°C) and 30 minutes and 2h (35±1°C), respectively. The higher temperature abuse hastens the colour changing response of TTI due to faster enzymatic kinetics with increasing temperature. Yan *et al*. (2008) [13] reported that the colour changing time of the indicator can be reduced by increasing enzyme content for indicators stored under same temperature.

3.2 Correlation of meat quality parameter with change in TTI colour during temperature abuse
Physico-chemical parameters

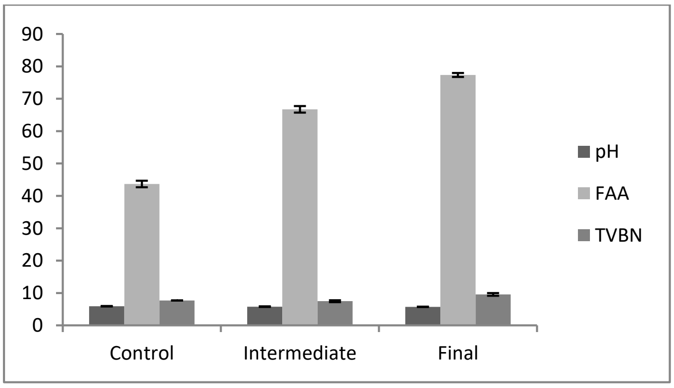
The pH value of meat changes with the exposure to different temperature (5±1°C, 15±1°C, 25±1°C and 35±1°C) and time combinations face during supply chain. In present conducted study, pH changes ranging from 5.92 to 5.79 during temperature abuse study with passage of time, which was significantly ($P<0.05$) decreased at 5±1°C, 15±1°C and 25±1°C due to accumulation of acidic metabolites produced during storage at intermediate and final colour alterations of TTI, whereas insignificant changes in pH was observed at 35±1°C due to short time interval to reach final colour of TTI (Figure 1). The decrease in pH of chicken meat during refrigerated stored poultry meat was also reported by Vaithiyanathan *et al.* (2008) [14] and Kumar *et al.* (2012) [15]. However, the decrease in the pH of the meat was not up to the extent which affects the meat quality attributes. Therefore, pH change of the meat at different temperatures abuse conditions are not significantly correlated with intermediate and final colour alterations of TTI.



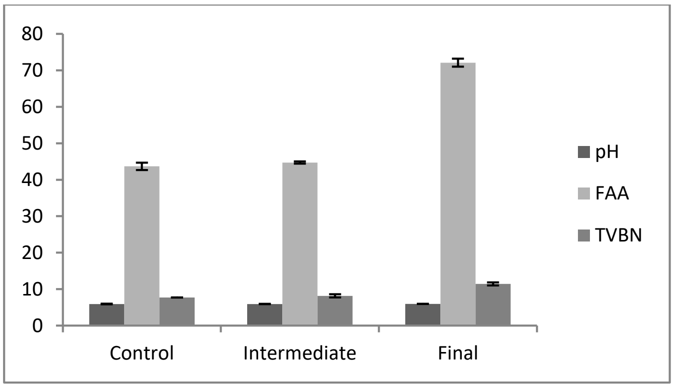
a) Changes in physico-chemical parameters at 5±1°C



b) Changes in physico-chemical parameters at 15±1°C



c) Changes in physico-chemical parameters at 25±1°C



d) Changes in physico-chemical parameters at 35±1°C
(Control: Urease based TTI kept at -18±2°C; Intermediate: Urease based TTI up to its intermediate colour; Final: Urease based TTI up to its final colour)

Fig 1: Changes in physico-chemical parameters in meat with urease based TTI colour response at different temperatures

Free amino acids (FAA) content of frozen chicken at different temperature abuse conditions clearly determine the quality and safety aspects during storage and supply chain. A significant ($P<0.05$) increase in FAA content of meat from 43.68 to 85.37 mg/100g was observed at different temperature abuse conditions (5±1°C, 15±1°C, 25±1°C and 35±1°C) suggesting breakdown of meat proteins by endogenous enzymatic action (Figure 1). However, the increase in FAA content of meat was within the prescribed meat safety standard (>120 mg/100g) at final colour alteration of TTI that clearly indicates absence of microbial spoilage in meat suggesting safe and wholesome for human consumption. Soni *et al*. (2018) [16] elucidated the increased free amino acids content in chicken meat from 61.53 to 126.93 during storage study of refrigerated chicken for seven days. Therefore, the changes in colour of TTI at intermediate and final stages are insignificantly correlated with FAA content of meat.

The release of total volatile basic amines (TVBN) occurs due to catalytic endogenous enzymatic action and microbial deamination of amino acids leads to production of various volatile amines. The changes in TVBN content were less significant ($P<0.05$) at different temperature abuse conditions (5±1°C, 15±1°C, 25±1°C and 35±1°C) ranges from 7.70 to 11.43 mg/100g (Figure 1). The TVBN content calculated were less than normal meat safety index (20 mg/100g) during final stages of colour alterations of TTI indicating absence of microbial spoilage and quality deterioration in meat thereby suggesting fit for consumption. Soni *et al*. (2018) [16] and Talukder *et al*. (2017) [17] also reported significant increased TVBN values for chicken meat stored under refrigeration but values did not exceed 20 mg/100g, which is indicative limit for deterioration (Rosen, 1957) [9]. Hence, the colour alterations of TTI are not significantly correlated with TVBN values of meat.

Microbiological parameters

Microbiological parameters were also performed at different temperature abuse conditions (5±1°C, 15±1°C, 25±1°C and 35±1°C) in terms of aerobic plate count (APC) and psychrophilic count. A slight increase in APC and psychrophilic count of meat was observed at different temperature abuse conditions ranging from 3.49 to 4.06 and 2.29 to 2.78 log_{10} CFU/g of meat, respectively (Table 1). The observed APC and psychrophilic count of frozen poultry meat were within the prescribed meat safety limits suggested by FSSAI (2017) [20] recommending safe and assured for consumption. An increasing trend in APC of aerobically packaged poultry meat from 4.60-6.38 log_{10} CFU/g during storage at 4ºC for 4 days was reported by Zhang *et al*. (2012) [18]. Huang *et al*. (2011) [19] also reported increase in total psychrophilic count of 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 9th day. Similarly, Talukder *et al*. (2017) [17] and Soni *et al*. (2018) [16] also described increasing trend in APC and psychrophilic count in the course of refrigeration storage study of chicken while correlating the colour changes in plant based TTIs and quality indicators. However, the increase in microbial load observed was not significantly correlated at intermediate and final colour alteration of urease based TTI.

Instrumental colour analysis

Colour of meat is most significant parameter determining consumer appeal, freshness and acceptability of meat and meat products. This colour can be estimated in terms of redness (a value) and yellowness (b value) of product thereby giving significant background regarding the overall factors controlling the quality and safety during supply chain. At different temperature abuse conditions (5±1°C, 15±1°C, 25±1°C and 35±1°C), the redness (a value) was significantly decreases whereas yellowness (b value) of the chicken meat samples was significantly ($p<0.05$) increases as compared to control at intermediate and final colour alteration of urease based TTI (Table 1). However, at 15±1ºC and 25±1ºC, redness (a value) value of chicken meat samples decreased significantly ($P<0.05$) and yellowness (b value) increased significantly ($P<0.05$). This might be due to temperature exposure causing loss of pigment in drip loss and meat became dull due to decreased colour intensity. However, there was no remarkable change in a value and b value of chicken meat kept at 5±1ºC and 35±1ºC. As refrigerated storage period of meat increases, metmyoglobin accumulation in meat tissue increased consistently causing decreased redness and increased yellowness (Sahoo and Anjaneyulu, 1997) [21]. The decreased a value and increased b value during storage were also reported by Soni *et al*. (2018) [16] and Talukder *et al*. (2017) [17] whereas Saucier *et al*. (2000) [22] observed a significant increase in b value in stored poultry meat. Therefore, the changes in instrumental colour analysis and colour alteration stages of TTI are insignificantly correlated.

Table 1: Changes in meat quality parameters with TTI colour response at different temperatures

Parameters	Microbiological characteristics (in log_{10} CFU/g)		Instrumental colour analysis	
	APC	Psychrophilic count	a value	b value
Initial Colour at -18±2°C (Control)	3.49 ± 0.04^{c1C1c2C2}	2.29 ± 0.04^{c1C1c2C2}	2.90 ± 0.10^{a1A1c2B2}	2.98 ± 0.18^{a1B1c2A2}
Intermediate Colour at 5±1°C	3.79 ± 0.02^{b1}	2.47 ± 0.03^{b1}	2.73 ± 0.02^{b1}	3.05 ± 0.06^{a1}
Final Colour at 5±1°C	3.90 ± 0.02^{a1}	2.64 ± 0.05^{a1}	2.58 ± 0.03^{b1}	3.20 ± 0.04^{a1}
Intermediate Colour at 15±1°C	3.66 ± 0.03^{B1}	2.41 ± 0.03^{B1}	2.73 ± 0.02^{B1}	3.23 ± 0.03^{A1}
Final Colour at 15±1°C	3.78 ± 0.02^{A1}	2.65 ± 0.02^{A1}	2.58 ± 0.03^{C1}	3.33 ± 0.03^{A1}
Intermediate Colour at 25±1°C	3.67 ± 0.03^{b2}	2.75 ± 0.03^{b2}	2.51 ± 0.03^{b2}	2.35 ± 0.08^{b2c2}

Final Colour at 25±1°C	3.96 ± 0.01^{a2}	2.91 ± 0.01^{a2}	3.10 ± 0.04^{a2}	3.26 ± 0.03^{a2b2}
Intermediate Colour at 35±1°C	3.83 ± 0.02^{B2}	2.47 ± 0.03^{B2}	2.95 ± 0.04^{A2}	2.58 ± 0.03^{B2}
Final Colour at 35±1°C	4.06 ± 0.01^{A2}	2.78 ± 0.02^{A2}	2.70 ± 0.03^{A2}	3.13 ± 0.03^{A2}

*Mean ± S.E. with different superscripts in row vary significantly (P <0.05) with respect to Control (-18±2°C) at different temperatures. a1, b1, c1 for 5±1°C treatment; A1, B1, C1 for 15±1°C treatment; a2, b2, c2 for 25±1°C treatment; and A2, B2, C2 for 35±1°C treatments

4. Conclusion

From aforementioned discussion, it was investigated that colour alteration at intermediate and final stages of urease based enzymatic TTI had not shown significant correlation with physico-chemical, microbiological and instrumental colour attributes of frozen chicken meat at 5±1°C, 15±1°C, 25±1°C and 35±1°C. Hence, the urease based enzymatic TTI has lesser applicability for monitoring thermal abuse of frozen poultry meat during supply chain. Therefore, it is concluded that urease based enzymatic TTI cannot be utilized for monitoring thermal abuse at various temperatures for different class of meat and meat products.

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