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Research Paper

Development and Evaluation of an Enzyme Based Time Temperature Integrator (TTI) for Monitoring Meat Quality

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ABSTRACT

A colorimetric enzyme-substrate based time temperature integrator (TTI) with potential for the development of intelligent packing tool, as a quality monitoring system for fresh meat under temperature abuse, is described. This biological TTI, which contains enzyme α-amylase and substrates iodine and starch, can produce a visible colour change on exposure to Ã 10°C. A ready to use TTI preparation protocol was standardized with respect to substrate and enzyme combinations, drying temperature and quantity of water required. Various constant temperature (°C) regimes of 4±1, 25±1 and 37±1 was followed for standardization of frozen (-18±1°C) fabricated TTI's. A colour response spanning from initial bluish black to final light yellow was observed on isothermal storage experiment. The rapid sequential colour response at 37±1°C compared to 25±1°C and unresponsiveness of TTI at 4±1°C explains temperature dependent kinetics of the present biological TTI. At last a colour chart was developed to compare the TTI's colour response with fresh meat quality under various constant temperature abuse conditions.

Keywords : *Time temperature integrator, Intelligent packing, Fresh meat, Temperature abuse, Colour change*

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INTRODUCTION

Indian livestock population is huge enough with potential to cater its non-vegetarian consumers. Even though, we have huge production capacity with respect to high quality raw material availability and efficient man power, our contribution to the global value chain of meat and meat products are scarce. The major existing drawback identified was insufficiencies in clod chain logistics for livestock products which may lead to fluctuations in temperature either during transportation or storage and ultimately affects the product's shelf life by enhancing microbial growth. This inadvertent failure in post-harvest management and fortuitous food spoilage creates great economic losses, by way of reduced product sale to producers at one end and by creating extra monitory burden for health care allied with food poisoning to consumers on the other end. Moreover, the advantages of new and sophisticated packaging systems are sacrificed under temperature fluctuations during transport or in display cabinet. In this side, temperature fluctuations can affect the anaerobic environment in a vacuum packed product or can modify the gas composition of a modified atmospheric packed product through altered/enhanced microbial multiplication (Limbo *et al.* 2010).To avoid whatever form of meat spoilage in supply chain and any additional harm to eventual consumers, a measure has to built-in in supply chain to manage temperature. Moreover, with increased legitimate stringency in hygiene and safety issues associated with fresh and processed meat products, it is of great importance to develop an intelligent packaging system/tool to monitor temperature abuse all the way through the supply chain.

Food quality assurance systems warrant a continuous monitoring and control of critical parameters like temperature throughout the supply chain for perishable food commodities. In most cases the conditions during transportation and at the retail level are out of manufacturer's direct control (Tsironi *et al.* 2008). Temperature is a critical parameter which is often deviating from the specifications and is one of the major factors influencing the rate of microbial development in foods (McMillin 2008) which can ultimately lead to food safety issues. So, an efficient system that can monitor any changes in predetermined temperature throughout supply chain or one that can detect exposure of food above a particular temperature is a need to check food spoilage in supply chain. Intelligent packaging, despite providing the established packaging functions, is equipped with supplementary functions such as detecting, sensing, recording, tracing and communicating thus facilitates consumer's decision making together with enhancing safety and quality of packed food products (Yam *et*

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al. 2005). Such packaging aids mainly comprise biosensors (Sankaran *et al.* 2011) and time–temperature indicators (Maietal 2011).Time temperature integrators (TTIs) are simple inexpensive devices that can attach to the package surface and integrate the cumulative effect of temperature exposure history of foodstuff (Taoukis and Labuza 1989) on its quality and safety. The response of the TTI should be clear, continuous and irreversible.

TTIs can produce an easily measurable response indicating the cumulative effect of time and temperature on product quality from the point of manufacture to consumption (Giannakourou *et al.* 2005; Nuin *et al.* 2008). Consumers can easily verify the food quality using TTIs response, which may be a colour development or a colour movement and that correlate to the left-over shelf life of a food stuff at any target temperature (Kerry *et al.* 2006). TTIs are applied to reflect the time- temperature history of the chilled and frozen foods such as marine food products (Tsironi *et al.* 2008) and meat and poultry products (Ellouze and Augustin 2010).

Commercially TTIs works on principles like diffusion as in Monitor Mark of 3 M Company (Kerry *et al.* 2006), enzymatic such as VITSAB Check Point (Tsironi *et al.* 2008), polymer based such as Fresh Check of Temp Time company (Nuin *et al.* 2008), solid state reaction represented by the On Vu™ TTI produced by the Ciba company (Tsironi *et al.* 2008) and microbiological systems such as TRACEO and eO of French company CRYOLOG (Ellouze *et al.* 2008). Among these, biological TTIs based on enzymatic reactions have been commercialized and well established in terms of theoretical interpretation. It is suggested that any TTI device with activation energy within 25kJ/mol from the food quality factor of interest could be acceptable to use for the prediction of food spoilage.

The aim of the study was to develop an enzyme-substrate based TTI and to evaluate its response at different storage temperature so as to check its suitability as a quality and safety monitoring device for fresh meat.

MATERIALS AND METHODS

Development of time-temperature integrator (TTI): An enzyme substrate based time temperature integrator using α-amylase on iodine starch clathrate complex was developed. The substrate for integrator was produced by mixing soluble starch and iodine solution. Various combinations of starch and iodine were evaluated to optimize the composition of substrate suited to different exposure temperature. Different substrate drying temperatures were evaluated for the milling of substrate into powder form. The time temperature integrator was prepared by mixing the powdered substrate and the enzyme. Various levels of enzyme were evaluated to select the optimum enzyme concentration suitable for the temperature of storage study. The level of water for incorporating into the enzyme substrate complex was also standardized for activating the TTI system.

The integrator was standardized in terms of substrate combination; drying temperature and enzyme level for evaluating the integrator response at isothermal storage temperatures. The time temperature integrators were fabricated and packed in 2×2 cm LDPE bags. The ready to use TTI was frozen at -18°C to avoid the action of enzyme. Proportion of substrate, drying temperature of substrate, enzyme concentration in TTI, quantity of water added to TTI and quantity of TTI packed are concealed for patent.

Evaluation of the TTI response at different constant storage temperatures: Storage temperatures similar to supply chain breakdown temperature were simulated in the laboratory in an incubator. The temperature conditions were monitored by using probe thermometer (Digi-thermo, WT-2, China). The ready to use time temperature integrators were kept at storage temperature of 25±1°C for 6, 12 and 18 h and 37±1°C for 4, 8 and 12 h. One control sample was kept at 4±1°C. The various storage temperature and storage time used for the evaluation of the developed TTI is given below:

C: Frozen at -18°C for 24 h and stored at 4±1°C

T1, T2 & T3: Frozen at -18°C for 24 h, exposed to 25±1°C for 6 h, 12 h and 18 h, respectively

T4, T5 & T6: Frozen at -18°C for 24 h, exposed to 37±1°C for 4 h, 8 h and 12 h, respectively. Multiple observations were recorded to validate the TTI response.

RESULTS AND DISCUSSION

Effect of substrate combination, drying temperature and enzyme level on the response of developed time temperature integrator (TTI): Various substrate combinations, drying temperatures and enzyme concentrations were standardized before the preparation of ready to use TTI. At any constant enzyme concentration, the substrate combination was found to be critical in determining the speed of colour changing reaction. A low concentration of iodine solution in substrate or higher starch content in substrate was found to be associated with a rapid change in TTI colour when exposed to room temperature for sufficient duration. Similarly, a high iodine concentration or low starch in substrate was associated with a slow reaction rate. Based on repeated experiments, a proper substrate combination was standardized which shows a moderate

reaction speed when mixed with enzyme and exposed to various storage temperatures.

The bluish black colour (after addition of water) of the TTI was due to a clathrate compound produced between starch and iodine. And this colour was directly proportional to the degree of polymerization of starch (Yan *et al.* 2007). The enzyme used in the present TTI, hydrolyzed this complex to produce a light yellow coloured end product when exposed to higher storage temperature for sufficient duration. So there should be equilibrium between the number of clathrate complex formed and number of enzyme molecules used in the TTI. This explains the importance of proper substrate combination in the development of TTI.

The substrate was dried at three different temperatures for a constant period of time and the dried substrate was milled using a mortar and pestle. The temperature of drying was standardized based on the easiness of powdering and fineness of the powder obtained. The drying temperature and duration used in present study gave optimum result in terms of easiness of powdering and fineness of the powder prepared.

The enzyme concentrations in TTI are equally important as of exposed storage temperature in determining the speed of the colour changing reaction. Different levels of enzyme in the TTI were evaluated by exposing the TTI at a particular storage temperature till end of reaction. Different time durations were noticed with different enzyme concentrations. Low level of enzyme failed to respond even after 12 h exposure at 37±1°C. But higher enzyme concentrations responded well in less than 12 h duration at 37±1°C. The enzyme concentration used in ready to use TTI in the present study showed a moderate colour change at 8 h and a complete colour change at 12 h of exposure at 37±1°C. At any particular storage temperature, the time required for the complete colour change of TTI was found to be directly related to the enzyme concentration.

Effect of isothermal storage temperature (37±1°C and 25±1°C) on colour response of the developed time temperature integrator (TTI): The ready to use TTI showed a gradual colour change from its initial bluish black colour to a final light yellow colour when exposed to any temperature above 10°C. The TTI colour response at different exposure temperature was compared with a control TTI kept at 4±1°C. It was noted that the colour changing response of the TTI was faster at higher temperature. The developed TTI did not show any visible colour change up to 6 h of storage at 37±1°C. A moderate change in TTI colour was noticed in 8 h of exposure at 37±1°C. After 10 h of exposure at 37±1°C, the colour change of TTI was more than that at 8 h exposure. And a complete colour change to a final light yellow colour was noticed in the developed TTI after 12 h exposure at 37±1°C.

At 25±1°C, the ready to use TTI showed a slow rate for colour changing reaction compared to 37±1°C. The TTI remained unchanged in the first 6 h of storage at 25±1°C. A moderate perceivable colour change was observed in the TTI only after 12 h of exposure at 25±1°C. And after 18 h of exposure at 25±1°C, the TTI completely turned to the final light yellow colour. Based on observations with respect to the colour response of the TTI under isothermal storage condition, a colour chart was developed. Simultaneously, experiments were conducted on fresh meat under similar temperature abuse conditions in the same laboratory. By comparing results of both experiments, it was concluded that the TTI response can be used to take decision regarding the quality of temperature abused meat.

The present study revealed that, the colour response of the developed enzymatic TTI related to both isothermal storage temperature and duration. The enzyme in the TTI got activated above 10 °C. The activated enzyme hydrolyzed the starch in the clathrate complex. This hydrolysis was responsible for the TTI colour change from bluish black to light yellow at higher temperature. The final light yellow colour of the exposed TTI was due to the light yellow colour of the amylase enzyme used during preparation of TTI. The speed of enzyme reactions varied with temperature and enzyme concentration and possibly this may be the reason for different TTI responses at different isothermal storage temperatures.

This has been supported by the finding of Yan *et al.* (2007) that the longest colour changing time of the indicator can be reduced by increasing enzyme content for indicators stored under same temperature. The applicability of the developed TTI in food system can be established with the concept of activation energy for different food spoiling reactions. The research from Labuza and Kamman (1983) reported that the indicator can be used in foods if the difference between the activation energy of various food spoiling reactions and activation energy of indicator was less than 40 kJ/mol. An activation energy value of 102-110 kJ/mol was established for amylase-starch system on various enzyme concentrations (Yan *et al.* 2007). Labuza and Kamman (1983) reported an activation energy value of 83.68-251.04 kJ/mol for microbial growth, 83.68-125.52 kJ/mol for nutrient loss during food quality losses. Hence the difference between the minimum activation energy needed for the integrator and various food spoiling reaction

was less than 40 kJ/mol, this integrator can be suitably used in food systems.

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