Development and characterization of a new calorimetric based Time Temperature Indicator

A

Thesis report submitted to

Thapar Institute of Engineering and Technology, Patiala

In the partial fulfillment for the

Degree of Master in Biotechnology

Submitted by:-

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UNDER THE GUIDANCE OF

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January - July 2019

DECLARATION

I **Tarun Sucheta**, do hereby declare that the work entitled **"Development and characterization of new colorimetric based time temperature indicator"** submitted to Thapar Institute of Engineering and Technology (T.I.E.T), Patiala, India, is a result of the investigation work carried out by me at CSIR-Central Food Technological Research Institute (CFTRI), Mysore, under supervision of **Rajeshwar S. Matche (Guide),** Senior Principal Scientist, Food Packaging department, CSIR-CFTRI, Mysore and **Prof. Dr. Sanjai Saxena (Co-Guide)**, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, during the period of January to July 2019.

Date- 01/07/2019

Place- Mysore

Tarun Sucheta

Signature of the Candidate

(TARUN SUCHETA)

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CERTIFICATE

This is to certify that the project work entitled **"Development and characterization of a new calorimetric based time temperature indicator"** in partial fulfillment of the requirement for the award of the degree **Masters in Biotechnology** from **Thapar Institute of Engineering and Technology (T.I.E.T), Patiala, Punjab, India,** is a record of the original research work done by **Tarun Sucheta.** He has carried out this work under my supervision at the **Department of Food Packaging Technology, CSIR-Central Food Technological Research Institute, Mysore,** during January to July 2019.

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Date - 10/07/2019

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Tarun Sucheta

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ABBREVIATIONS

Sr. No.	Abbreviation	Full-Form
1.	TTI	Time Temperature Indicator
2.	E_a	Activation Energy
3.	LAB	Lactic Acid Bacteria
4.	MAP	Modified Atmosphere Packed
5.	CO_2	Carbon Dioxide
6.	AMB	Aerobic Mesophilic Bacteria
7.	PTFE	Polytetrafluoroethylene
8.	TVBN	Total Volatile Basic Nitrogen
9.	TVB	Total Viable Bacteria Counts
10.	M	Molar
11.	ul	Micro-litre
12.	R^2	Coefficient of Determination
13.	KJ/mol	Kilo Joule per mole
14.	RMSE	Root Mean Square Error
15.	FSSAI	Food Safety And Standard Authority of India
16.	WTO	World Trade Organization

EXECUTIVE SUMMARY

Food and consumer safety is primary objective of any food industry; hence to achieve this important objective, food quality as well as food and consumer safety should be maintained from production to final consumption where food spoilage is controlled. Food spoilage is the result of combine effect of two crucial factors i.e., time and temperature. The visible color changing time temperature indicator (TTIs) are usually monitoring the food spoilage by the correlation of activation energy (Ea) of both TTI and food spoilage parameters. The characterization of color changing TTI strip where the concentration of TTI substrate i.e., lactic acid and activation energy (Ea) of the color response (from pink to yellow) are addressed. The activation energy of each developed TTI strip was calculated by plotting an Arrhenius curve between 1/T where T is absolute temperature and *ln* k. The correlation coefficient of determination R^2 and activation energy (Ea) of each TTI are described. Among all TTI developed, five TTIs showing the higher coefficient of determination R^2 as well as activation energy ranged from 25.64 to 121.48 KJ/mol that can be suitable for various range of food products. The developed TTI can be used for chicken meat and ground beef as the previous studies reported that the activation energy values of ground beef, cooked chicken meat as well as raw chicken meat are ranged between 84 KJ/mol to 115 KJ/mol. Chicken meat spoilage analysis was performed by considering the TVB (Total viable bacteria counts) and TVB-N (Total volatile base nitrogen). The activation energies of the TVB-N production and TVB level during the storage of chicken meat were 44.29 KJ/mol to 63.74 KJ/mol. The MATLAB curve fit tool (*cftool*) was used to apply different linear and nonlinear regression models. Based on the individual model summary, the correlation coefficient of determination R^2 and %RMSE were selected for the optimization of the regression model as least %RMSE and higher R^2 value show the goodness of fit for the particular regression model. The derived activation energy can be considered as targeted activation energy and applied in the regression model to acquire the concentration of TTI substrate for the developed TTI strip. By the regression model, the TTI substrate concentration was calculated, i.e., 0.45M. Therefore, 8µl of 0.45 M lactic acid was used as an activation substrate of the developed TTI strip and employed on the chicken packet for real-time monitoring of the chicken meat spoilage.

Keywords:- Time temperature indicator, Lactic acid, activation energy, %RMSE, TTI strip.

CHAPTER – 1

INTRODUCTION

1. INTRODUCTION

The crucial role of Packaging is to protect the Food products from decaying so that the food industries can adapt it to extending the shelf life and simplify the processing of their goods in the external environments. Packaging comes in market with having various sizes and shapes and is made up of different materials. The essential function of the packaging of the product is to exceed the quality, protect, convenience, communication with consumer, containment, preservation.

Packaging gets modernized with time due to changes in the distribution practices and globalization of food business depends upon the prolonged shelf life of the product so as to accommodate distance and complex transport (Schaefer, D. and Cheung, W.M., 2018) Innovations in packaging required to increase the functionality, so as to fulfil the additional demands of the consumer.

Technology developments often need coverage for packaging innovation to be adopted. These have to include developments in transportation, transport infrastructures, post-harvest technology, new retail formats, domestic appliances such as refrigerators, freezers and microwave ovens. Also, also the socio-cultural and demographic trends, consumer lifestyles and economic climate must generate sufficient market demand for innovation to succeed (Richard Coles, 2003).

1.1 Development of Packaging

From Past 200 years, it has seen the pack evolve from being a container for the product to becoming an essential element of entire product design. An overview of some developments in packaging during the past 200 years is:

1. Introduction

1800-1900

- In 1809, thermally preserving food in hermetically sealed jars gets produced in France.
- In 1810, Peter Durand designed the soldered tinplate canister and commercialized the use of heat preserved food container.
- Development of paper bag making machine (Davis,1967).
- Albert L. Jones in USA patented the use of corrugated materials for packaging (1870s).
- In 1879, Robert Gair of New York produced the first machine-made folding carton.
- The 1880s, Quaker Oats packaged the first cereal in a folding box (Opie,1989)
- The Crown cap for glass bottles and fully automatic bottle making had commercialized in 1899 (Robertson, 2002).

1901-1950

- Paraffin wax coated paper milk containers in Los Angeles was introduced in 1906.
- In 1912, Regenerated cellulose film (RCF) was developed
- In 1915, John, Toledo, Ohio commercialized the paper bottle, a folded blank box called Pure pack which further used in dairy.
- The use of frozen foods in packed form was commercialized in 1920s in New York. 1930s, American brewers began selling canned beer.
- Ethylene and Polyethylene was first introduced by ICI Ltd. which extensively used in packaging in the 1940s.
- In 1943, Aerosol can be developed and was used for dispensing food products such as Pasteurized processed cheese and spray dessert toppings.

- In 1946, PVdC (Polyvinyldene chloride) was used as a moisture barrier resin.
- In 1950s, Retort pouch for heat processed foods was developed.
- In 1956, Aluminium trays, cans and squeezable plastic bottles and tetra packs launched its trihedral milk cartons that were constructed from LDPE extrusion.
- The two-piece drawn and wall ironed (DWI) developed for carbonated drinks and beers.
- In 1967, the ring pull opener was drawn up for canned drinks by MB company.
- The TBA and UHT cartons have become one of the major packs for liquid and beverage

1951-2000

- Boil in the bag frozen meals and Map retail was introduced in 1970s.
- PVC was used for beverage bottles, frozen foods in microwaveable plastic containers
- FFC and PET bottles were developed in 1973.
- In 1980s, Co-extruded plastics incorporating oxygen barrier plastics materials squeezable sauce bottles and microwave heated bottles.
- PET coated bottles for ready to meals.
- In 1990s, Digital printing on carton sleeves and labels for food packaging was introduced which more widely adopted by the drink companies sought ways of better brands differentiating their.

(Data collected from Rooney, M.L., 2005)

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1.2 The value of packaging to society:

Value of Food packaging to society has never been greater. In response, stakeholders in the food industry need to appreciate fully and actively promote the positive contributions that their packaging makes to the quality of life. Food packaging is governed by a mass of laws, codes of practice and guidelines (Realini, C.E. and Marcos, B., 2014). The benefits are:

- Reduce or eliminates the risk of tampering and adulteration.
- Present food in a hygienic and often aesthetically attractive way.
- Reduces product damage and food spoilage, thereby saving energy and vital nutrients and protecting the health of the consumer.
- Provides Functional convenience in use or preparation, freeing up more time.
- Promotes goods in a competitive marketplace and increase consumer choice.
- Extends the shelf life with the benefit of prolonged product use, thereby reducing wastage.

However, the primary functions of packaging are more explicitly stated:

- Containment: depends on the product physical form and nature.
- Protection: prevention of mechanical damage due to hazards of distribution.
- Preservation: prevention or inhibition of chemical changes, biochemical changes and microbiological spoilage.
- Information about the product: legal requirements, product ingredients, use etc.
- Convenience: for the pack handlers and users throughout the packaging chain.
- Presentation: material type, shape, size, colour, merchandising, display units etc.
- Brand communication: pack, symbols, illustrations, advertising and color, thereby creating visual impact.
- Environmental responsibility: in the manufacture, use, reuse, or recycling and final disposal(RichardColes,2003).

-

1.3 Packaging Standards and Specifications

The packaging assessment must include a definition of optimum quality standards and these standards should not be compromised by cost. Ideally, packaging supplier selection is a techno-commercial decision between the purchasing function and packaging technologists. Buyers are becoming more discerning and now expect suppliers to have a quality assurance schemes that are accredited by a third party. Widely used Quality Management Systems (QMS) are those based on ISO 9000.

Quality assurance on production and packaging lines has been facilitated by the use of integrated computerized micro-electronic control systems that can detect a range of defects and automatically eject rejected packs. For example, there are automatic check weight, metal detectors, fill-level sensors, pack leak detectors, pack dimension sensors, light transmission sensors and odour detectors.

The packaging specifications for a national brand, however, may require packaging that needs to provide a higher degree of protection due to the wide variation in storage conditions and distribution hazards experienced during packs of delivery through a several retailer's distribution channels. Thus, the rigours of the distribution system and lack of control over it by food manufacturers often lead to specifications are geared to ensuring that a very high percentage of products arrive in a pristine and safe condition. However, this approach of packaging conflicts with the significant pressure to minimize packaging.

The primary testing methods for materials are available from a wide range of sources including ISO, American Society for Testing and Materials (ASTM), British Standards Institute, Bureau of Indian Standards (BIS) etc.

Some traceability system should be implemented across the world to ensure the safety and authenticity of the food during supply chain (food industries, retailers, consumers). The new challenges unfold day by day, which gives scientists a reason to discover a new packaging solution against it. The types of technologies of packaging system are as follows:-

1.3.1 Active packaging

It is the method used for packaging, which gets introduced as a novel concept in response to the continuous changes in the consumers' needs and market trend. The innovations and applications of packaging get advanced so that it provides protection and longer shelf life (Kerery et al., (2006). This technology implants some components into the packaging to release or absorb some substances which are helpful in sustain the quality of the product with longer shelf life, i.e., ethylene scavengers, antioxidants, oxygen scavenger, antimicrobial agents, moisture regulators, release/absorption of flavours and odour. With this technology, there is a reduction in the localisation activity and movement of the particles from the film to food.

1.3.2 Intelligent packaging

The term Intelligent involves an ON/OFF switching function on the package in response to changing the external/internal stimuli, to communicate the product status to its consumers (Dobrucka and Cierpiszewski., 2014).

It is capable of carrying out intelligent functions such as sensing, detecting and tracing recording and communicating specific types of information (Otles and Yalcin., 2008). Accordingly, intelligent packaging systems consist of hardware components such as time-temperature indicators (TTI), gas detectors, freshness or ripening indicators and Radio Frequency Identification (RFID) systems.

1.3.2.1 Principle of intelligent packaging

In packaging, "smartness" can have many meanings, and covers a number of functionalities, depending on the product being packaged – food, beverage, pharmaceutical, household products etc. Examples of current and future functions that are considered to have "smartness" would be packages that (Pavelkove, A., 2013):

1. Retain the integrity and actively prevent food spoilage (extend shelf life).

2. Enhance product attributes (look, taste, flavour, aroma, etc.).

1.3.3 **SMART PACKAGING**

The concept of intelligent packaging (also described as smart packaging) is to sense some of the properties of the food it encloses or the environment in which it is kept and can inform the manufacturer, retailer, and consumer of the state of these.

It is different from the concept of both active packaging and intelligent packaging can be used to check the effectiveness and integrity of the active packaging systems describe a package is “intelligent” if it can track the product, intellect the environment inside or outside the package, and communicate with the consumer. For example, an intelligent package is one that can monitor the quality/safety condition of a food product and provide early warning to the consumer or food manufacturer.

Intelligent packaging refers to a package that can sense environmental changes, and in turn informs the changes *(*Pavelková, A., 2013*)* to the users defined intelligent packaging as having two categories: simple, intelligent packaging, and interactive or responsive intelligent packaging.

Fig 1.1 - Functions of Packaging (Kuswandi et al., 2011

1.4 TIME-TEMPERATURE INDICATOR

It is an economical device that can attach externally to the package surface of the product which reflects fully or partially the time-temperature history of the foodstuff. Earlier, TTI not used in the food industry. It was mainly functional in reflecting the time-temperature history of chilled or frozen food which was sensitive to temperature, like seafood, milk, frozen fish. TTI was also used to review the sterilisation process, such as thermally processed milk (Claeys et al., 2003).

Based on the working principle, TTI's subdivided into three types: i) chemical based system, ii) biological based system and iii) physical based system. Therefore, it is necessary that TTI response matches the quality degradation, must fulfil for the successful application of TTI's of the food products. The activation energy (E_a) of temperature sensitive TTI (Taoukis and Labuza, 2003) response correlated with activation energy (E_a) of quality losses of the food product should matches (Wanihsuksombat et al., 2010).

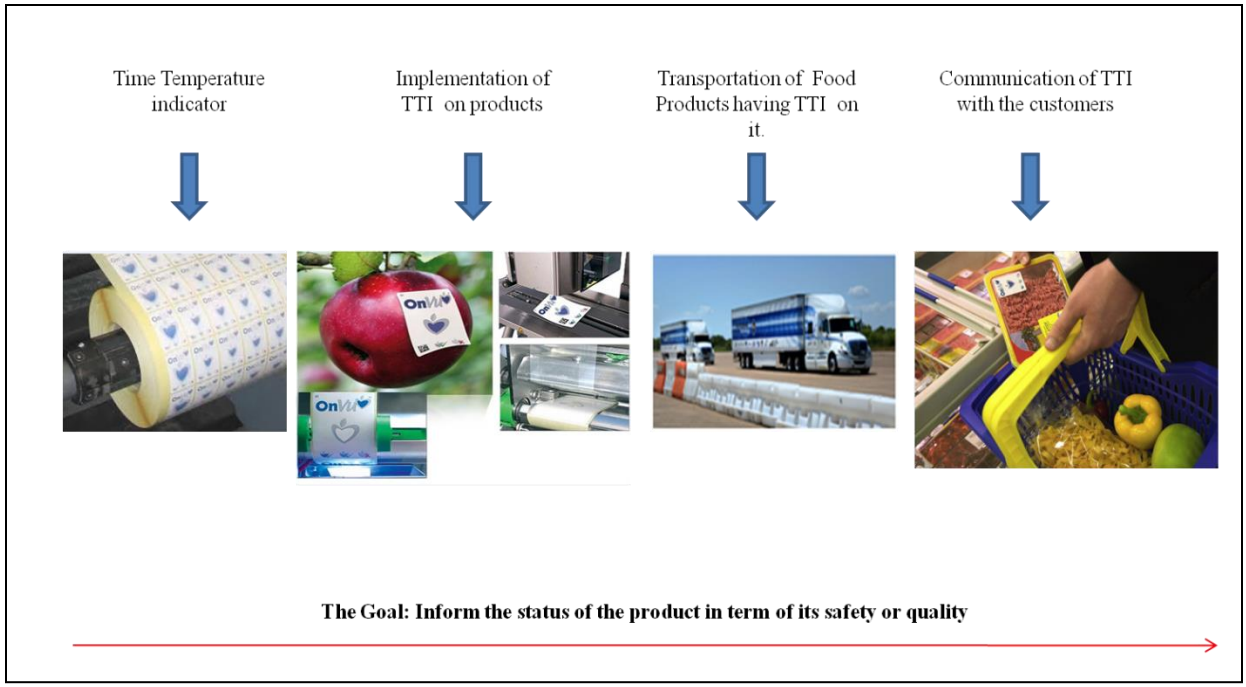


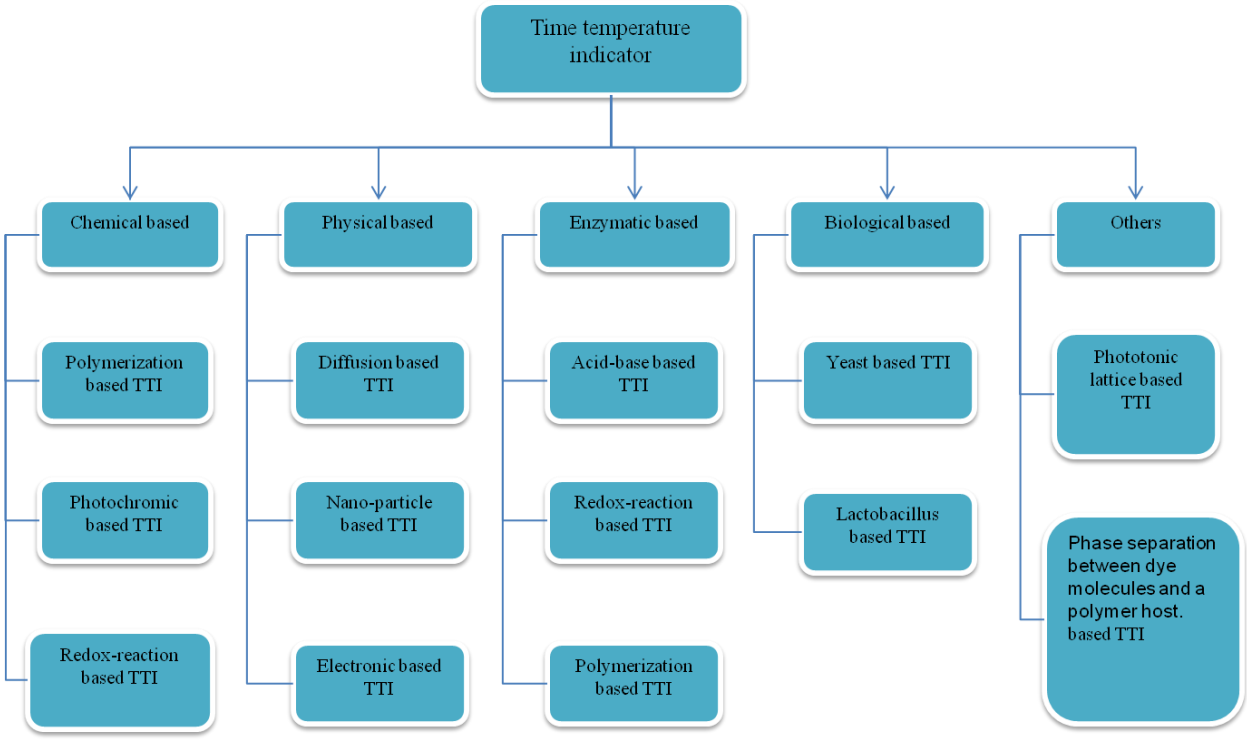
Fig 1.2 - Goal of Time-Temperature Indicator

CHAPTER – 2

REVIEW OF LITERATURE

The applications of time-temperature indicator technology have brought about significant advancement in food quality and safety control, based on the patent of Midgley in 1993. Throughout distribution and storage of food products, temperature management is also an essential factor in the quality loss. With the appropriate temperature management during storage and transportation be able to deliver high-quality food products. There are many types of TTI's classified which are working on different principle had shown below:-

2.1 Classification of Time-temperature indicator:



TTI's allow indicating the remaining shelf-life of perishable products and the recording thermal history through the colour change by the accumulative effect of time and temperature (Wang et al., 2015). Moreover, (Taoukis and Labuza, 2003; Pavelkove, 2013) reported that TTIs are cost-effective and user-friendly devices to monitor, record, and translate the overall effect of temperature history. TTI's generally categorised as chemical, physical and biological based on the principles they make use of to achieve colour change. TTI's subdivided into three types:

2.1.1 Physical-type diffusion-based TTIs: Wanihsuksombat et al., (2010) reported that lactic acid-based TTI prototype based on the vapour diffusion of lactic acid could be applied to show the time-temperature history of some foods. (Kim et al., 2016) studied that a prototype isopropyl palmitate (IPP) diffusion-based TTI system showed potential for monitoring the microbial quality of non-pasteurized angelica (NPA) juice based on temperature abuse.

3M Monitor Mark® *(3M Co., USA)* is a diffusion-based indicator label and indicated by temperature-dependent permeation and a blue-dyed fatty acid ester diffusing along with a wick. The response range of this TTI is 48 hours for -15°C, 48 hours for 5°C, 48 hours for 10°C, and one week for 31°C (Pavelková 2013; Wang et al. 2015; 3M United States 2017). Time strips® (Timestrip UK Limited, UK) uses a special porous membrane. The squeezing of a start button leads to move from the liquid to directly contact the membrane, and then the liquid diffuses through the membrane (Kuswandi et al. 2011). Time strips response range is limited to 2 hours - 7 days with an available temperature range of -20 to 30°C (Timestrip, patent no.US7232253B2). Physical-type diffusion-based TTIs would be problematic because of the exudation of the colour material and the fact that ageing porous substances would cause an adverse impact on safety and accuracy (Wang et al., 2015).

2.1.2 Chemical-type TTIs: Ohta et al., (2008) reported that a colour indicator based on the bacterial strain (sk22) isolated from commercial cod developed for confirmation of temperature abuse in the cold chain. The colour in this indicator changed for 32-72 hours at 12°C. Kim et al. (2012) studied a laccase based TTI prototype could be applied to predict losses of food quality ascribed to enzymatic changes, hydrolysis, and lipid oxidation. Murakami et al., (2012) reported that a simple indicator developed using red cabbage dye with sodium hydrogen carbonate, sodium carbonate and lactose and changed for 36, 48, and 96 hours in 12, 10, 4°C, respectively.

Kuswandi et al., (2013) showed that a novel on-package colour indicator based on bromophenol blue could be used for real-time visual monitoring of freshness state of packaged guavas through the colour indicator gradually changed colour from blue to green after five days on room temperature. Yamamoto and Isshiki (2012), developed Maillard reaction-based TTI.

This TTI was useful of chilled temperature distribution and has validated the accuracy of colour change for alerting the growth of *Listeria monocytogenes* (Rokugawa and Fujikawa, 2015) (Park et al., (2013) reported that the laccase-based TTI, including NaN3, was composed two parts of an enzyme solution and a substrate solution and could predict the Pseudomonas fragile growth.

As the commercially available TTIs, Fresh-Check® TTI (Temptime Co., USA) is using highly coloured polymer by a solid state polymerisation reaction. The polymer gradually darkens on the colour that tends to reflect the cumulative exposure to temperature. If the inner colour is darker than the outer colour, it means that the product has reached the end of shelf-life. The indicator needs to kept at -24°C before application as indicators could be activated above the storage temperature (Pavelková 2013; Wang et al., 2015). However, the range of use gets limited due to the storage temperature. Other disadvantages is the potential toxicity of polydiacetylene compounds. The accuracy is affected by the selected compound and the presence of sunlight or bright direct light, which can accelerate the polymerisation reaction (Wang et al., 2015).

OnVuTM TTI (Ciba Specialty Chemicals & Fresh point Inc., Switzerland) a solid state reaction TTI, is based on the colour change of photosensitive compounds and organic pigments by temperature. Additionally, this TTI gets activated by the UV irradiation, changes from colourless to blue. The rate of change of colour is proportional to temperature (Pavelková 2013; Wang et al., 2015). The flexible using for various foods limited because of the fixed rate of change in an identical temperature.

2.1.3 Biological-type TTIs: Vaikousi et al., (2009) reported that the colour change in a microbial TTI prototype based on the growth and metabolic activity of *Lactobacillus sakei*_strain was similar with the lactic acid bacteria (LAB) growth in fresh ground meat stored under modified atmosphere packed (MAP) conditions. Nopwinyuwong et al., (2010) studied that a novel colourimetric indicator label for monitoring the freshness of intermediate-moisture dessert spoilage used pH-sensitive dyes, bromothymol blue, methyl red, and carbon dioxide (CO2), and the indicator response correlates with microbial growth patterns.

Kim et al., (2012) reported that a microbial TTI using the Weissella cibaria CIFP 009 (psychrotrophic lactic acid bacterium) could be predicted accurately about aerobic mesophilic bacteria (AMB) counts, lactic acid bacteria (LAB) counts, and freshness.

The commercially available TTIs, Check Point® TTI (Vitsab A. B., Sweden) based on a colour change by the enzymatic system. The pH gets decreased by controlled enzymatic hydrolysis of a lipid substrate, and the pH decrease has occurred the colour change of a pH indicator from deep green to bright yellow to orange-red (Kuswandi et al., 2011; Pavelkove, 2013). eO® TTI (CRYOLOG, France) is based on pH change by controlled microbial growth selected strains of lactic acid bacteria and is stored in a frozen state of -18°C to prevent the bacterial growth. The colour of this TTI is changed to red by temperature abuse, or when the product reaches its use by date (Pavelkove, 2013). Ellouze and Augustin (2010) reported that it could be used successfully as a quality management tool for meat products. However, it considered that flexible using for various foods gets limited due to the difficulty of rate control for microbial growth.

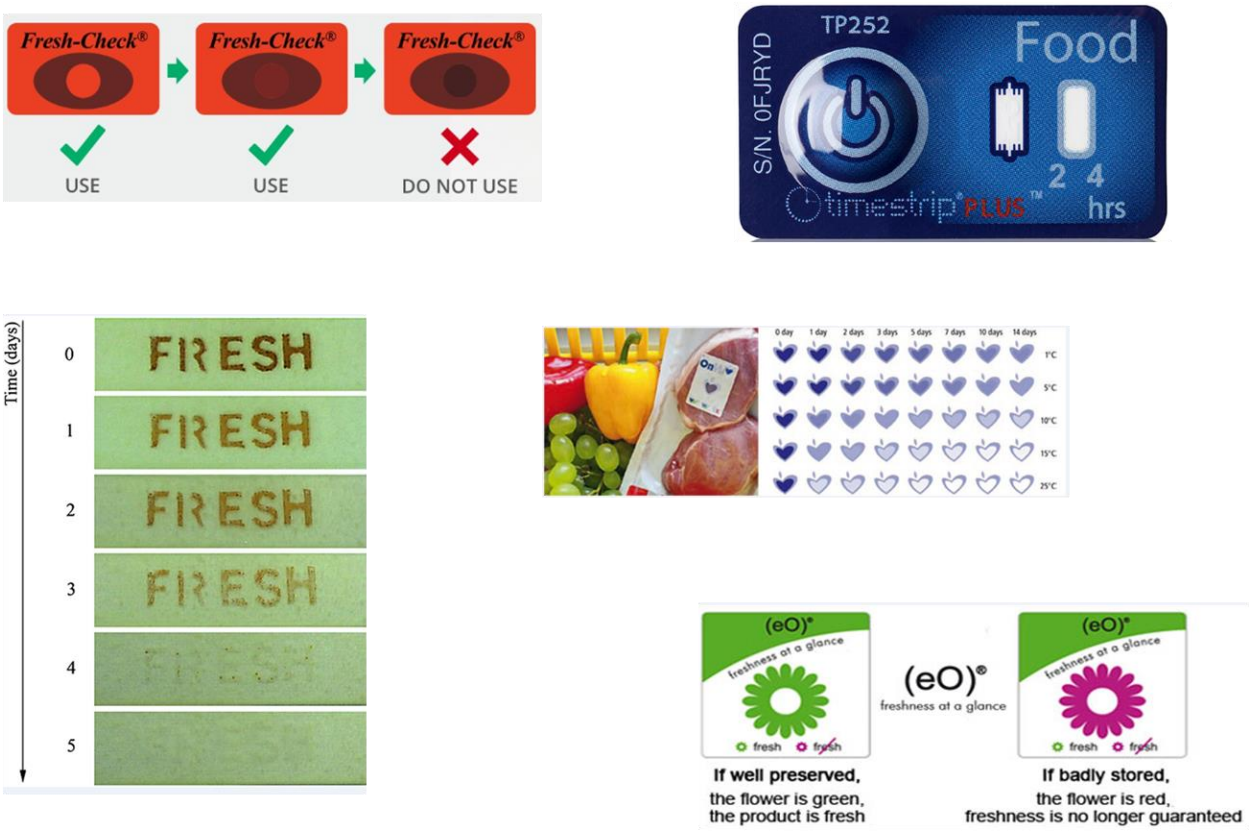


Fig 2.1 - Commercially available TTI's

Table 2.1-Types of TTIs with different working and experimental methods:

Types	Principle	Working	Example	Experimental	References
Chemical based	Polymerisation based TTI	Monomer and polymer of diacetylene can change the colour during polymerisation and rate of polymerisation and the rate of polymerisation increase with temperature and time.	Fresh-Check®	A mixture of PVC and polyvinyl acetate used as an ink for the thermochromic label (PDA material) which can record thermochromic transition photographically in the range of 20-65°C.	Phollookin et al., (2010).
			HEATmarker®	A crystallised diacetylene compounds like alkylurea (2,4-hexadiyn-1, 6-bis) with solvent (to dissolve) used in TTI which irreversibly show changes in colour as the environment changes.It directly depends on the solvent system in which the colour change is related to the solubility of the diacetylenic monomer in the solvent system when temperature and time change.	Baughman et al, (2009).
				The acetylene compound (2,4-hexadiyn-1,6-diethyl urea) get synthesised by propargylamine and isocyanate, which also shown colour changes from white to blue, and even to black as the polymerisation rate increases.	Song et al, (2013).
				The formation of TTI based on PDA vesicle with having an amphiphilic polymer solution which monitors the temperature range 0–50°C.	Qian et al., (2012)
	Photochromic based TTI	It is a thermally induce fading reaction process in which photochromic compound shows different colours after activation at different wavelengths. The fading process change with the accumulation of time and temperature.	OnVu™	New photochromic liquid- carbosilane dendrimer developed in which the thermal back-isomerisation reaction rate k_H is much smaller than photochemical backisomerization positive/reverse reaction rate k_t/k_c.	Zhang et al., (2005)
				To form H-aggregation, a spiropyran derivative attached to the side chains of polyacrylates or polysiloxane which shows the absorption spectrum had a blue shift. Other factors such as polarity of the solvent, compound property, the pH, metal ions of the solution affect the rate of thermally induced fading reaction. The thermally induced close-loop reaction of the modified open-loop spiro aromatic slows down obviously, and the reaction duration may be extended.	Krongauz et al., (1988)

				Another spiro-pyran thio alkyl or aryl thio is a kind of derivative that is challenging to be bleached by ambient light but also has high stability and printability when stored at room temperature before its activation.	Salman et al., (2010)
				The colourless or slightly coloured spiro aromatic compound in the thermodynamically stable state and the coloured compound in the meta-stable state. When the colour changes to a pre-determined meta-stable state, the light induction can be removed to prevent photobleaching.	Tenetov et al., (2012)
				The different spiro-pyran compound identified and studying its photochromic reaction under UV radiation at different temperatures to confirming that either it can be used as a quality indication for cold chain products at low temperature or not.	Kreyenschmidt et al., (2010)
				Diarylethene is a compound based on valence isomerism. It converts the open loop or valence isomer in the form of valence isomerism. The open loop more stable in thermodynamically form while closed loop is in coloured or metastable form.	Levy et al., (2007)
	Redox-reaction based TTI	It is light-induced redox-reaction based TTI, which shows a colour change when the compound comes in contact with air, and it gets react with the oxygen which is directly related to the accumulation of time and temperature.		The derivative of anthraquinone used in printing the ink which gets decomposed into crimson particles. When the particles react with the oxygen, the colour starts changing, which get influenced by both time and temperature.	Galagan et al., (2008)

Physical-based	Diffusion-based TTI	It based on diffusion where the diffusion of the coloured chemicals, i.e., fatty acid esters, phthalates, specific polymers through the porous wick which made of specially designed material, from one reservoir to another.	Monitor Mark™	TTI which is working based on dyes diffusing through a dye compatible polymer composition when the polymer composition is above the determined temperature.	Ezrielev et al., (1995)
			Tempix TTI	The viscoelastic material migrates into a diffusely light-reflective porous matrix at a rate that varies with temperature to change the light transmissivity of the porous matrix progressively and thereby provide a visually observable indication.	Arens et al., (1997)
				The amorphous material in used contact with a porous matrix.	Spevacek et al., (2003)
				The dyeing chemical material with low-temperature melting point moves along the capillary/ porous diffusion tube.	Ye et al., (2004)
				Printable TTI which could be printed directly on the substrate materials or at the interlayer between substrate materials. This indicator includes a protective layer, a diffusion layer, two reagents on the substrate or the protective layer or the diffusion layer, an optional outer protective layer and an optional external base layer.	Koivukunnas et al., (2008)
				The volatile property of materials. In the process (heat–evaporation–adsorption) of real transformation, the colour gets changes. During the storage of food, the volatile dye is absorbed by the absorption layer after heating, and the number of volatile dyes positively correlated with the cumulative thermal effect. It targeted at specific heat-sensitive products, the kind and amount of dyes can be adjusted in combination with other methods to control the rate of dye evaporation, so that the colour response of TTI can be consistent with the food quality.	Deng et al., (2013)

	Nano-particle based TTI	Nanomaterial used, which has a perfect thermochromic property.		The silver-based nanoplates used as a thermochromic material. After absorbing heat, the surface morphology of Ag nanoparticle changes, which results in the shift of wave numbers to a visible region.	Zeng et al., (2010)
				The gelatin-templated AuNPs (gold particle) kept at 30°C at different times, and they stored at -20°C. Distinct colour development can be observed in as early as six hours at 30°C, and the colour intensity is proportional to the duration of exposure.	Lim et al., (2012)
				Kinetically programmable Ag overgrowth on Au nanorods. In the reaction of epitaxial overgrowth of Ag shell on Au nanorods, which shows a sharp colour change, which can be dynamically adjusted.	Zhang et al., (2013)
	Electronic-based TTI	The thermal sensor that converts temperature signals to electrical signals and then converts electrical signals to final visual output.		The electronic TTI includes a high-frequency temperature logger to collect information that will pass to a computing device that is a microprocessor, and then the food's thermal history will recorded. It is used to monitor the quality of insulin.	Zweig et al., (2005)
				The electronic label which includes a temperature sensor and an oscillator. When the external temperature of food changes, the temperature sensor transmits the information to the oscillator, and then the frequency of oscillator changes. There is a counter in the TTI that records the complete cycles of the oscillator.	Jensen et al., (2013)
				A conductor layer, an insulator layer, a transparent conductor layer and a doped polymer layer in which the dopant can be an acid, base, light latent acid or light latent base. It gets activated when the temperature reached a particular value, the acid or base component will start corroding the conductor layer, which changes the conductor layer's electrical. The change can visualised by using ink containing metal particles as the conductive layer, which means that the TTI can utilise without the support of professional equipment.	Haarer et al., (2012)

Enzymatic based	Acid-base reaction based TTI	The lipid substrate is hydrolysed by lipase in controlled conditions, which results in a decrease of pH. Consequently, the colour of the pH indicator changes. The rate hydrolysis of the lipid substrate directly proportional to the temperature. The continuous colour change can also be measured, and thus, the product quality can be estimated by observing the colour change.	CheckPoint™	An enzymatic TTI prototype (xylanases) that used in the temperature range of 100−130°C.	Gogou et al., (2010)
				The urea substrate, phenol red indicator, urease solution, disodium hydrogen phosphate–potassium dihydrogen phosphate used as a buffer solution. The buffer solution gets mixed in order to ensure catalytic reaction occurs in a specific range of pH. Urease catalyses the decomposition of urea to produce ammonia, which causes pH to change and consequently brings about the colour change of the phenol red indicator.	Wu, (2005)
				The components of the system are alkaline lipase solution, mannose solution, Gly-NaOH solution also, bromine thymol blue solution (pH indicator).	Lu et al., (2012)
	Redox reaction based TTI			New enzymatic TTI based on laccase, in which the components are laccase solution, guaiacol (substrate for laccase), bovine serum albumin (enzyme stabiliser) and sodium acetate buffer. Laccase catalyses guaiacol oxidation discolouration to indicate the cumulative time-temperature exposure.	Kim et al., (2012)
				Developed a glucoamylase-type TTI, in which glucoamylase catalyses both the hydrolysis of dextrin and iodine solution works as the indicator. The extent of a colour change indicates the cumulative time-temperature effect.	Qian et al., (2012)
				Based on starch and amylase based reaction in which blue substance gets formed when iodine solution reacts with starch.	Cai et al., (2006)

Biological-based	Yeast-based TTI	Yeasts and lactic acid bacteria have been widely used in biological TTI systems recently. Other bacteria such as Streptococcus can also be available in the TTI systems.	TRACEO® eO®	The TTI system based on the anaerobic respiration of yeast to generate acid in certain circumstances, especially at a specific temperature, resulting in the colour change of the pH indicator. The first part contains the reactants, including microorganisms and colour indicator made of aqueous ink with appropriate carrier separating them, both of which are attached to a transparent plastic film; the second part comprises an activator with an aqueous adhesive layer, and it gets activated when the reactant contact with the activator.	Varlet-Grancher et al., (2006)
	Lactobacillus-based TTI	The lactic the acid generated by lactic acid bacteria to change the pH under certain conditions, which leads to a colour change to indicate the accumulation effect of time and temperature.		It is a two-dimensional code containing the colour information of the TTI that changes with mutative time and temperature; people can judge the quality of the product quickly by scanning the two-dimensional code to obtain the colour information.	Lee et al., (2013)
Others TTI	TTI based on phototonic lattice change			Based on the photonic crystal (PC-TTI), which consists of a substrate, a mesh layer, a photonic crystal patterned film and a hardener pouch. The photonic crystal in the patterned film shows the correlation between band-gap shift and temperature, thus providing a visible indication of the range of temperature.	Chen et al., (2013)
	TTI based on thermochromic polymer/dye blends			Based on the phase separation between dye molecules and a polymer host. Polymers with built-in threshold temperature sensors exhibit a change of their absorption characteristics in response to external heat.	

CHAPTER – 3

SCOPE OF THE STUDY

3. SCOPE OF THE STUDY

Existing TTIs have a limitation on flexibility at a range of reaction temperature or reaction period. Since storage characteristics of each kind of food are very different even at the same storage temperature, ideal TTIs should have flexibility corresponding to each food characteristics by adjusting the reaction rate. However, most of the existing TTIs do not have flexibility control the variation rate in the same temperature and broaden available temperature range. TTI colour changes need to be correlated to food quality and safety changes to provide additional information on food distribution under the selected temperature conditions.

Therefore, the aim of the investigation was colour changes induced under various conditions and establish a predictive model for estimating the time required for these changes as a function of temperature and reactant colour variation characteristics.

Although so many efforts have been made to develop the diffusion-based colour changing, TTI and simultaneously the prototypes for the same were developed, but still, there is a lack of research for the development of the product based TTI. Hence the development and characterisation of paper-based, product base colour changing TTI.

CHAPTER – 4

OBJECTIVE

4. OBJECTIVE

1) Development of the paper-based, cost-effective TTI.
2) Characterization of TTI for monitoring the shelf life of perishable food products.
3) Validation of TTI on different food products.

CHAPTER – 5

MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1 Development of the paper-based, cost-effective TTI

5.1.1 Material

Cresol red (HiMedia, Mumbai, India) were used as pH sensitive dye indicators. Propanol (Merck, USA) was used as a solvent for the indicator. Lactic acid (Merck, USA) was used as a TTI substrate. Whattmann^{TM} filter paper No-1 (11 microns) was procured from GE Healthcare Life sciences, USA for the carrier of the indicator. PTFE hydrophobic membrane (1 micron) was supplied by SS Filters Pvt. Ltd, Mumbai, India.

5.1.2 *Methodology*

Cresol red and propane-2-ol were used to prepare a dye mixture solution in accordance with (Wanihsuksombat et al., 2010). An indicator solution was prepared with the concentration of 0.5% (w/v) cresol red was prepared in 50% (v/v) of propane-2-ol. The pH of the indicator dye adjusted to 9.8 to achieve a dark pink colour. Then, it gets printed on the Whatman paper. The mentioned TTI proto-type was employed to make commercial indicator strip which facilitates application on the food product. The TTI made as the description was activated with the different concentration of lactic acid. The PTFE hydrophobic membrane put in between the activator and the coloured indicator. The TTI get protected with the LDPE material and stick on food products with the help of pressure sensitive adhesive.

5. *Materials and methods*

Fig. 5.1 - Dye with pH 9.2-9.8

Fig. 5.2 – pH get adjusted by Eutech pH meter

Fig. 5.3 - Dye get printed on whatman filter paper by using Epson L360

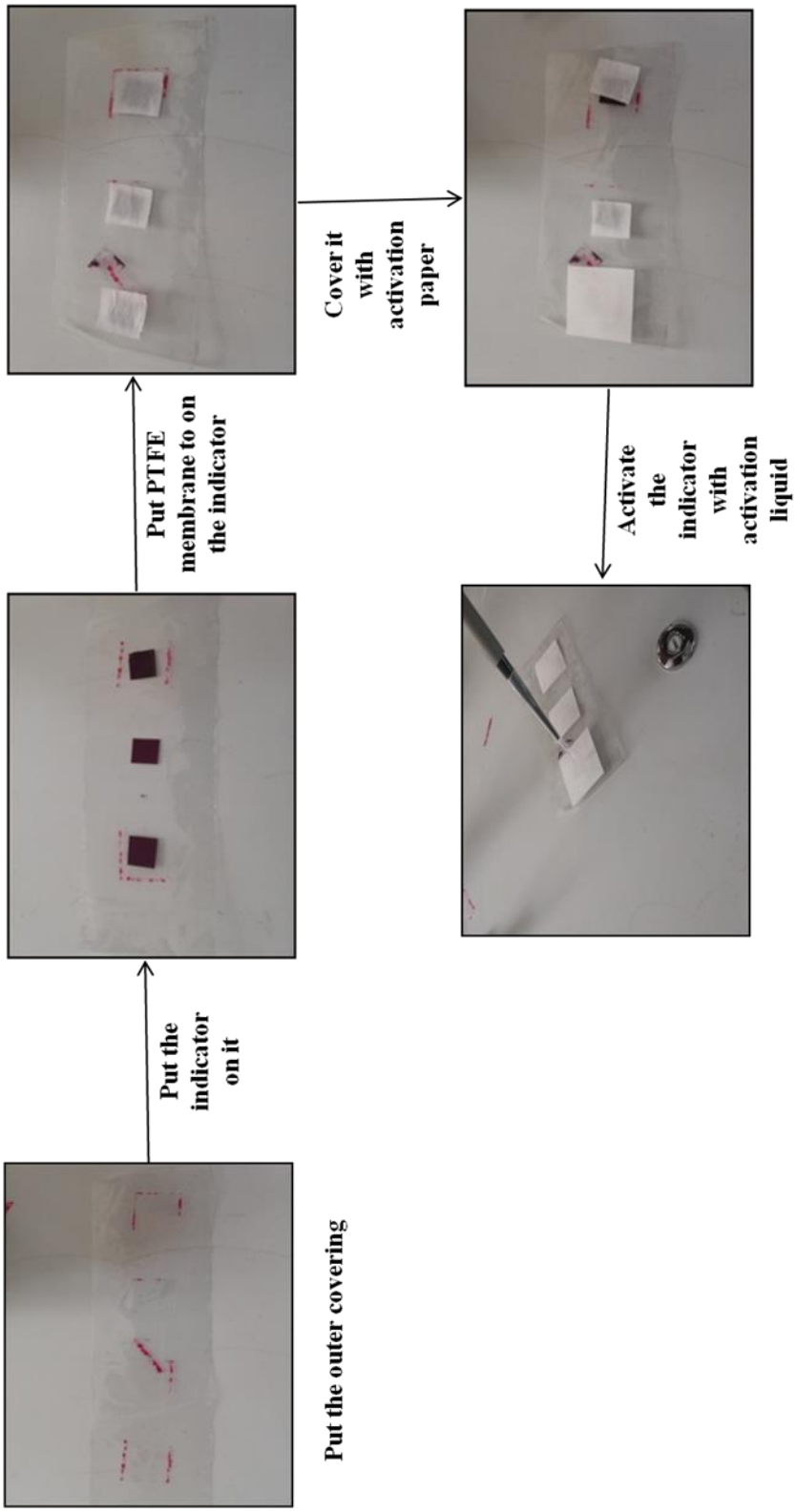


Fig: 5.4 Steps of preparation of lactic acid based TTI strip

5.2 Preparation of lactic acid based TTI strip

Dye mixture was printed on the Whattmann^{TM} filter paper No-1 (11 micron) by the ink tank printer (Epson L3110) for the even distribution of the dye mixture on the carrier. The printed paper was cut into 1×1 cm^2 pieces for the preparation of the TTI indicator strip. The TTI strip was developed by arranging the indicator paper, PTFE membrane and activation paper as described in Figure 5.2.

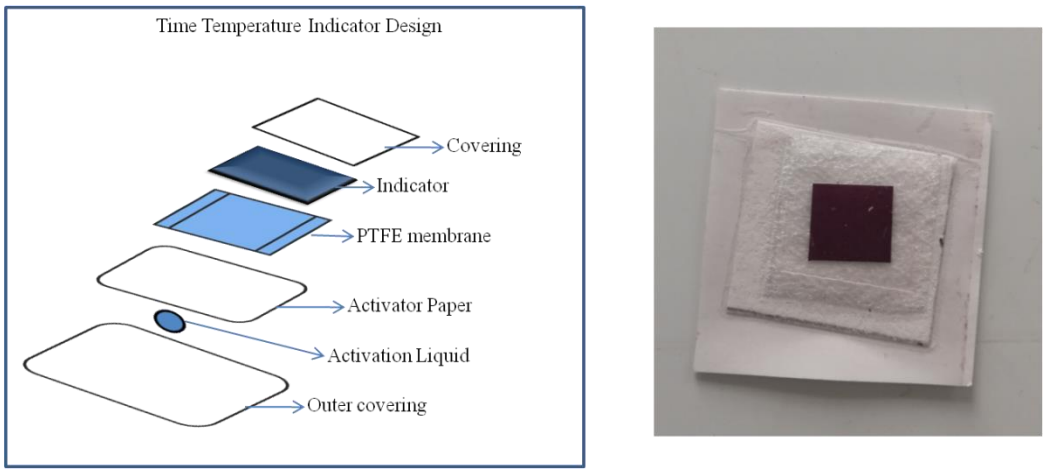


Fig: 5.5 - Design of developed lactic acid based TTI strip

Lactic acid was dropped on the activation paper in five different concentrations, i.e., 0.05M, 0.1M, 0.25M, 0.5M and 1M, i.e., as well as at three different volumes, i.e., 5µl, 8µl and 10µl, to check the effect of volume and concentration of TTI substrate (Table 5.1)

Table 5.1: Different types of TTI strips

Code	Concentration of TTI substrate (Lactic acid) (M)	Volume
#1	0.05	5ul
#2	0.1	5ul
#3	0.25	5ul
#4	0.5	5ul
#5	1.0	5ul
#6	0.05	8ul
#7	0.1	8ul
#8	0.25	8ul
#9	0.5	8ul
#10	1.0	8ul
#11	0.05	10ul
#12	0.1	10ul
#13	0.25	10ul
#14	0.5	10ul
#15	1.0	10ul

5.3 Determination of dynamic parameters of lactic acid-based TTI strip

The Hunter Color Lab (Konica Minolta, Japan) was used to estimate the corresponding values of L*, a* and b* of developed TTI strips. The total colour difference (ΔE) was calculated as a dynamic parameter for the colour response of TTI strips, according to Wanihsuksombat et al., (2010).

Fig. 5.6 – Color analysis (Konica Minolta)

The developed TTI strips were tested isothermally at three different temperatures i.e., 4±1, 27±2 and 37±4 ^{o}C which shows the refrigerated condition, ambient condition and accelerated condition of storage respectively. The TTI kinetics was established between the colour response and time up to the full-colour change of the TTI. The cumulative colour response X=ΔE was expressed in terms of a response function described by Yan et al., (2007) as well as Taoukis and Labuza (1989).

$$F(X) = kt \quad (1)$$

where k stands for the rate constant of the reaction, correlating with temperature as well as t indicates the storage time at which the TTI strip responses to colour change. By plotting the curve between the natural log of k (ln k) and 1/T where T is the absolute temperature of the storage, the activation energy of the individual TTI strip was calculated by following Arrhenius function:

$$\ln k = \frac{Ea}{RT} + \ln A \quad (2)$$

where R is considered as gas constant (8.314 KJ/mol) and A is Arrhenius constant.

5.4 Establishment of regression models

In order to reach the least sum of squares and optimise the regression model, curve fitting tool (*cftool*) from MATLAB was used. The activation energy of the TTI and concentration of TTI substrate were implicated for the goodness of fit based on the least sum of the square for various regression models described in Table 2.

Table 5.2: Regression models derived from MATLAB

Sr.no.	Regression Model	Equation
1	Linear	$Y = a + \mathrm{bx}$
2	Logarithmic	$Y = a + \mathrm{b}\ln \mathrm{x}$
3	Inverse	$Y = \mathrm{a} + \frac{b}{x}$
4	Quadratic	$\mathrm{Y} = \mathrm{ax}^2 + \mathrm{bx} + \mathrm{c}$
5	Cubic	$\mathrm{Y} = \mathrm{ax}^3 + \mathrm{bx}^2 + \mathrm{cx} + \mathrm{d}$
6	Power	$\mathrm{Y} = \mathrm{a}^{\mathrm{b}}\mathrm{x}^{\mathrm{b}}$
7	S	$Y = exp.(\mathrm{a} + \frac{b}{x})$
8	Growth	$Y = exp.[\mathrm{a} + (\mathrm{bx})]$
9	Exponential	$Y = a * [exp(\mathrm{bx})]$

5.5 Analysis of chicken spoilage parameters

To check the accuracy of the derived regression model, chicken meat was selected as model food where the spoilage study was conducted on the basis of total volatile basic nitrogen (TVB-N) and microbiological study (Total viable bacterial counts; TVB) (Kuswandi et al., 2014; Wang et al., 2017). The chicken meat was stored at three different isothermal conditions (4±1, 27±2 and 37±4^{o}C), and spoilage analysis was carried out which correlated to activation energy (Ea) of the chicken spoilage. According to the derived regression model the activation energy of the spoilage parameters was considered to get the concentration of TTI substrate. Furthermore, the known amount of TTI substrate was used to initiate the real-time monitoring of chicken by developed TTI strip where the activation energy was supposed to be taken additional, to maintain the food safety limit as described by (Taoukis and Labuza, 1991).

CHAPTER – 6

RESULTS AND DISCUSSION

6. RESULTS AND DISCUSSION

6.1 Effect of different concentration and volume of the TTI substrate (Lactic acid)

Developed time-temperature indicator strips were subjected to different concentration of TTI substrate, i.e., lactic acid with different volumes as described in Table 1. Each TTI strip was showing colour change after the particular time when it was stored under isothermal conditions (4, 27 and 37℃) (Table 3). The colour change from pink to yellow, of the TTI strip, was a gradual process concerning time and temperature where the diffusion of the TTI substrate plays a significant role. The diffusion of the lactic acid increases the concentration of H+ ions and ultimately changes the pH of the TTI which can be observed by visual colour change (Kerry et al., 2006; Courbat et al., 2009). TTI. Table 3 narrates that among all developed TTI strips, theTTI #15 is showing the fastest colour change based on the various isothermal condition which might be due to the higher concentration (1M) as well as higher volume (10µl) of the TTI substrate (Lactic acid). Similar results were observed by Kim et al., (2012) during the development of lactic acid bacteria based TTI.

Table 6.1 - Corresponding time of colour change by each TTI

Code	TTI Substrate Concentration (M)	Volume	37 degree (Hours)	27 degree (Hours)	4 degree (Hours)
#1	0.05	5ul	5	9-10	110-120
#2	0.1	5ul	4-5	8-9	96-110
#3	0.25	5ul	4	7-8	80-100
#4	0.5	5ul	3-4	6-7	70-90
#5	1.0	5ul	2	4-5	48-60
#6	0.05	8ul	4-5	8-9	100-120
#7	0.1	8ul	4	7-8	90-100
#8	0.25	8ul	3-4	6-7	72-96
#9	0.5	8ul	2-3	5-6	60-80
#10	1.0	8ul	1-2	3-4	36-50
#11	0.05	10ul	4-5	7-8	100-120
#12	0.1	10ul	4	7-8	80-100
#13	0.25	10ul	3-4	6-7	72-96
#14	0.5	10ul	2-3	5-6	60-72
#15	1.0	10ul	1-2	3-4	24-48

6.2 The dynamic analysis of developed TTI strips

By using equation (1), the regression lines were plotted between the colour response (ΔE) and respected time (t) taken for changing the indicator colour from pink to yellow, for developed indicators (TTI#1 to TTI#15). These response functions are linear with time and showing the dramatic colour change with storage time at particular isothermal conditions. The reaction constant k of all developed TTI at three different isothermal conditions (4, 27 and 37 ^{o}C) are listed in Table 6.1

Table 6.2 - Reaction constant of developed TTIs at different isothermal conditions

k value			
Code	**4 degree**	**27 degree**	**37 degree**
#1	0.6583	1.2767	2.0109
#2	0.3578	1.7066	2.8861
#3	0.2621	0.5374	1.0196
#4	0.4583	0.6275	1.1236
#5	0.5105	1.1249	2.0226
#6	0.4635	2.3341	2.4712
#7	0.2557	0.7217	4.4341
#8	0.2972	0.8961	4.0182
#9	0.4917	1.6307	2.0945
#10	0.5716	1.8258	3.1259
#11	0.6286	1.0960	3.4372
#12	0.7129	1.4932	3.8643
#13	0.7522	1.5120	3.9214
#14	0.7986	1.5567	4.2934
#15	0.8157	1.6472	4.4108

According to equation (2), the activation energy of each developed TTI strip was calculated by plotting an Arrhenius curve between 1/T where T is absolute temperature and *ln* k. The correlation coefficient of determination R^2 and activation energy (Ea) of each TTI are described

in (Table 6.3). Among all TTI developed, the TTI # 6, #7, #8, #9 and #10 are showing the higher coefficient of determination $R^{2,}$ i.e., 0.9973, 0.9929, 0.9914, 0.9957 and 0.9940 respectively, as well as activation energy ranged from 25.64 to 121.48 KJ/mol that can be suitable for various range of food products. Especially, the developed TTI can be used for chicken meat and ground beef as the previous studies reported that the activation energy values of ground beef, cooked chicken meat as well as raw chicken meat are ranged between 84 KJ/mol to 115 KJ/mol (Nulin et al., 2008; Vaikousi et al., 2009; Ellouze and Austin, 2010). Hence the TTI #6 to #10 are considered for the regression analysis, for the consideration of regression analysis between activation energy and concentration of TTI substrate (Lactic acid).

Table 6.3 - Correlation coefficient of deatermination and activation energy (Ea) of developed TTIs

Code	Concentration (molar)	Volume	Activation energy ($E_{a)}$	R^2
#1	0.05	5ul	21.32	0.9275
#2	0.1	5ul	36.41	0.9247
#3	0.25	5ul	27.37	0.9265
#4	0.5	5ul	34.68	0.9288
#5	1.0	5ul	45.22	0.9277
#6	0.05	8ul	25.64	0.9973
#7	0.1	8ul	37.12	0.9929
#8	0.25	8ul	59.85	0.9914
#9	0.5	8ul	86.47	0.9957
#10	1.0	8ul	121.48	0.9940
#11	0.05	10ul	26.62	0.9473
#12	0.1	10ul	49.40	0.9465
#13	0.25	10ul	36.27	0.9473
#14	0.5	10ul	82.61	0.9425
#15	1.0	10ul	95.43	0.9467

6.2 Optimization of the regression model for TTI strip

The activation energy (Ea) of TTI #1 to #15 were plotted against the concentration of TTI substrate to get the suitable regression model for calculating the concentration for target activation energy. The MATLAB curve fit tool (*cftool*) was used to apply different linear and nonlinear regression models as described in Table 5.2. Based on the individual model summary, the correlation coefficient of determination R^2 and %RMSE were selected for the optimization of the regression model as least %RMSE and higher R^2 value show the goodness of fit for the particular regression model (Table 6.4).

Table 6.4: Correlation coefficient of determination R^2 and %RMSE of various regression model derived from MATLAB

Sr.no.	Volume of TTI substrate (Lactic acid)	Regression Model	Coefficients				Model Summary		
			a	b	c	D	R^2	Adj.R^2	%RMSE
1	**5 µl**	Linear	0.034	-0.754	-	-	0.650	0.534	0.265
2		Logarithmic	1.031	-3.191	-	-	0.585	0.447	0.289
3		Inverse	-29.020	1.319	-	-	0.517	0.356	0.312
4		Quadratic	0.002	-0.089	1.165	-	0.765	0.530	0.266
5		Cubic	3.058E-5	-0.001	22.122	0.307	0.780	0.561	0.257
6		Power	3.222	3.250E-5	-	-	0.595	0.460	0.884
7		S	-96.033	1.633	-	-	0.590	0.453	0.890
8		Growth	0.102	-4.834	-	-	0.594	0.498	0.886
9		Exponential	0.030	0.031	-	-	0.594	0.458	0.886
10	**8 µl**	Linear	0.010	-0.228	-	-	0.942	0.938	0.090
11		Logarithmic	0.508	-1.642	-	-	0.805	0.790	0.165
12		Inverse	-18.949	0.806	-	-	0.592	0.561	0.238
13		Quadratic	6.280E-5	0.001	0.007	-	0.977	0.973	0.059
14		Cubic	4.876E-7	0	-0.006	0.117	0.978	0.972	0.060
15		Power	5.219	21.48	-	-	0.959	0.956	0.234
16		S	-69.980	0.099	-	-	0.841	0.829	0.461
17		Growth	0.029	-3.357	-	-	0.940	0.935	0.283
18		Exponential	0.030	0.031	-	-	0.959	0.946	0.280

19	**10 µl**	Linear	0.012	-0.305	-	-	0.817	0.756	0.192
20		Logarithmic	0.614	-2.044	-	-	0.727	0.636	0.234
21		Inverse	-27.062	0.964	-	-	0.615	0.486	0.278
22		Quadratic	0	-0.023	0.552	-	0.926	0.852	0.149
23		Cubic	8.974E-6	-0.001	0.064	-0.852	0.975	0.902	0.122
24		Power	1.965	9.763E-5	-	-	0.775	0.701	0.658
25		S	-91.765	0.506	-	-	0.736	0.648	0.714
26		Growth	0.036	-3.566	-	-	0.792	0.722	0.634
27		Exponential	0.036	0.028			0.792	0.722	0.634

6. Results and Discussion

As per the Table 6, the quadratic model and cubic model for the 8 µl TTI substrate (lactic acid), is suitable as best fit according to higher correlation coefficient of determination R^2i.e., 0.977 for the quadratic model and 0.978 for cubic model as well as lower %RMSE, i.e., 0.059 and 0.060 for the quadratic model and cubic model respectively as compared to other models have studied. Hence the optimised regression model (Equation 3 and 4) can be utilised to stimulate the developed TTI for the targeted activation energy (Ea). Hence the TTI strip can be used to monitor the spoilage/freshness of the particular food, irrespective to the other parameters.

$$Y = ax^2 + bx + c \quad (3)$$

$$Y = ax^3 + bx^2 + cx + d \quad (4)$$

Where *x*= Targeted activation energy (Ea) of the particular food

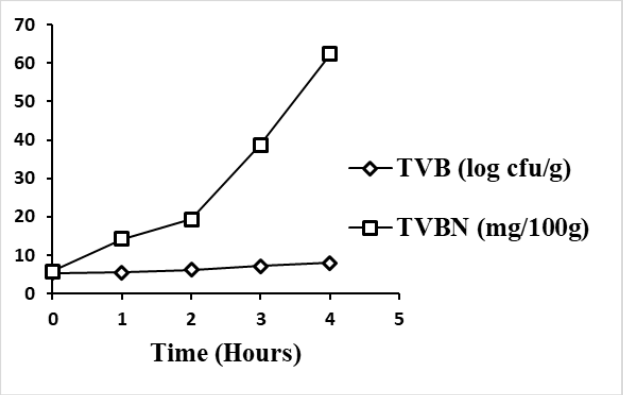
Y= Concentration of TTI substrate (Lactic acid)

a, b, c, d are the coefficients of the model

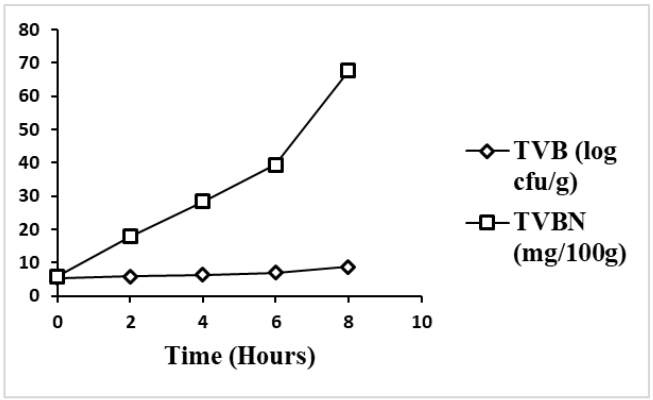
6.4 Validation of regression model by chicken meat analysis

Chicken meat spoilage analysis was performed by considering the TVB (Total viable bacteria counts) and TVB-N (Total volatile base nitrogen). Figure 1 described the TVB-N and TVB analysis of chicken meat samples at three different isothermal conditions, i.e., 4±1, 27±2 and 37±4°C. As per the results indicated that the total viable bacteria count (TVB) was increased with increase in storage period irrespective to storage temperature. The initial level of TVB in the chicken samples was 5.42 log cfu/g which is in the agreement of the range reported by the previous works i.e., 2 to 5 log cfu/g (Goksoy et al., 2004; Zhang et al., 2012; Ghollasi et al., 2017). During the storage study, the level of TVB has reached beyond the permissible limit described by Food Safety and Standard Authority of India (FSSAI, 2011) as well as World Trade Organization (WTO, 2015), i.e., 6 to 7 log cfu/g. As per Figure 1. the TVB-N is increasing with the increase in storage period irrespective to the temperature, which shows that the chicken meat was spoiled due

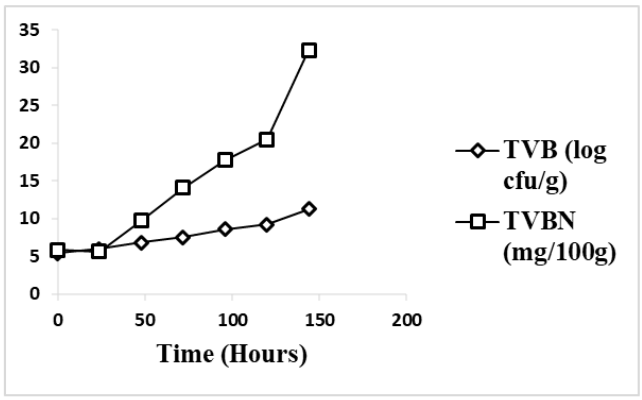
to the production of certain biological amines. Similar results were observed by Balamatsia et al., (2007) during the study of the role of possible volatile amines on the quality of the chicken meat. Kuswandi et al., (2014) was also observed an increase in the level of TVB-N during the real-time monitoring of the broiler meat quality.



Spoilage analysis of chicken meat at 37 ±4 °C



Spoilage analysis of chicken meat at 27±2 °C



Spoilage analysis of chicken meat at 4 ±1°C

Figure 6.1 - TVB and TVB-N analysis of the chicken meat samples at three different isothermal conditions i.e., 4 ±1, 27 ±2, 37 ±4°C.

The activation energies of the TVB-N production and TVB level during the storage of chicken meat were44.29 KJ/mol and 63.74 KJ/mol respectively, obtained by plotting the curve between absolute temperature (1/T) and ln k where k was rate constant of the TVB-N and TVB for different isothermal conditions. (Figure 6.2).

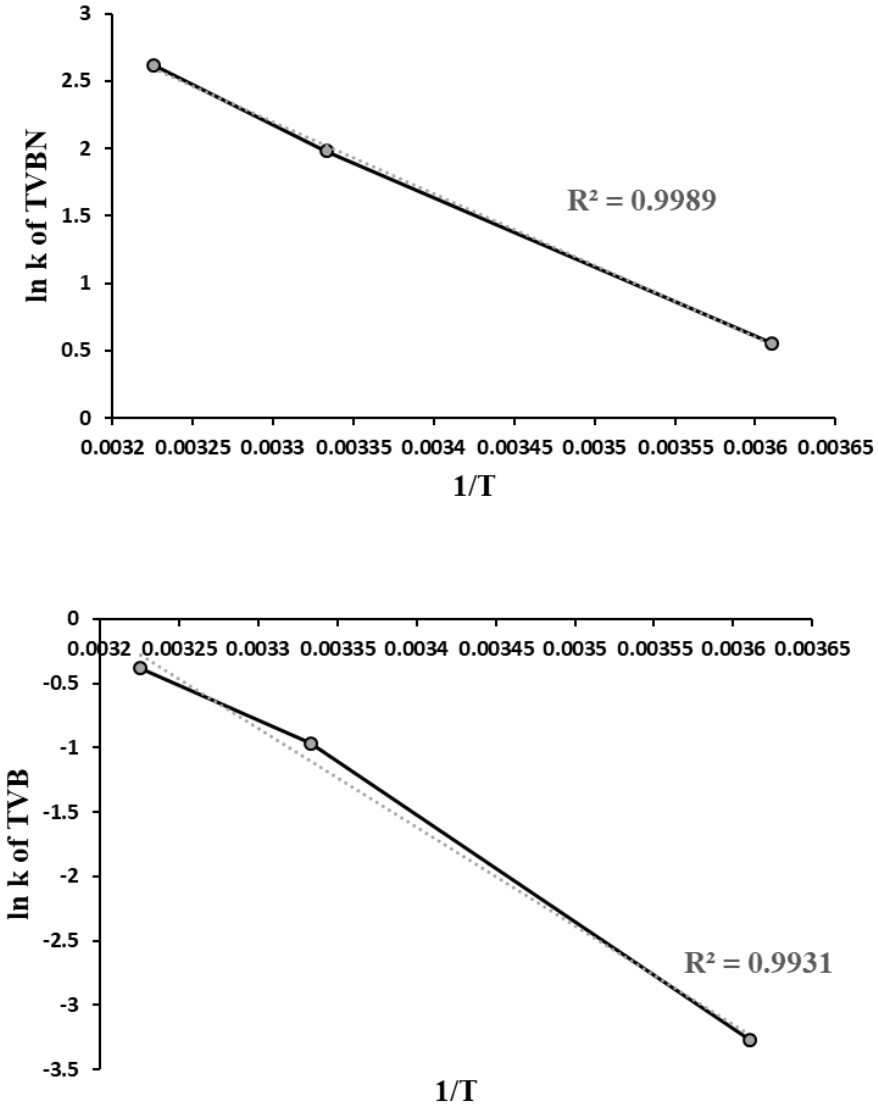


Figure 6.2 - Graph of 1/T vs ln k, for the calculation of activation energy of TVB-N and TVB

6. Results and Discussion

The derived activation energy can be considered as targeted activation energy and applied in the regression model to acquire the concentration of TTI substrate for the developed TTI strip. By the regression model, the TTI substrate concentration was calculated, i.e., 0.45M. Therefore, 8µl of 0.45 M lactic acid was used as an activation substrate of the developed TTI strip and employed on the chicken packet for real-time monitoring of the chicken meat spoilage (Table 6.5). The developed TTI is showing colour changes which correlate with chicken spoilage (Plate 6.1).

Table 6.5: Real-time Monitoring of the chicken meat spoilage at 4±1 ºC.

Time (Hours)	TVB (log cfu/g)	TVBN (mg/100g)	ΔE (8ul,0.45M)	Corresponding colour change
0	5.42	5.83	0	
24	5.97	5.94	21.24	
48	6.84	9.7	38.73	
72	7.55	14.12	56.8	
96	8.61	17.73	65.81	
120	9.2	20.53	64.82	
144	11.3	32.36	63.22	

Initial Dark purple color
(Fresh chicken)

Intermediate purple and orange color
(Immediate consumption of the chicken)

Orange color
(Spoiled chicken)

Plate 6.3 - Real-time monitoring of the spoilage of chicken meat

CHAPTER – 7

CONCLUSION

7. CONCLUSION

In the present research, the lactic acid-based TTI strip was developed. The relationship between the activation energy (Ea) of colour changing TTI and concentration of lactic acid as an activation liquid was considered, for the establishment of a suitable regression model by MATLAB (*cftool*). The quadratic regression model was selected based on goodness of fit parameters, which is useful to find the concentration of TTI substrate, activation liquid (lactic acid) for the targeted activation energy of the particular food. The spoilage analysis of chicken meat was performed to check the accuracy of the developed TTI by monitoring the real-time freshness or spoilage based on TVB-N values. The developed lactic acid-based TTI strip is easy to handle and applicable on any perishable food products.

The present research focused on the development and characterisation of lactic acid-based TTI strip, which can be useful for any perishable food product by considering the activation energy (Ea) of particular spoilage parameter. There is a still strong research gap for the establishment of the particular mathematical tool for the development of internal spoilage indicator based on the activation energy (Ea) of the spoilage parameter of particular perishable food which facilitates the accurate determination of spoilage of the food product without activation substrate. This type of prototype may solve the major issues related to food and consumer safety.

CHAPTER – 8

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