Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2017) 41: 496-505
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doi:10.3906/vet-1603-93

Development and evaluation of time-temperature integrator for monitoring high temperature thawing of frozen buffalo meat

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Received: 29.03.2016 • **Accepted/Published Online:** 10.06.2017 • **Final Version:** 21.08.2017

Abstract: Research was undertaken to develop an enzyme–substrate-based time-temperature integrator (TTI) for monitoring higher temperature thawing of frozen buffalo meat. Alpha-amylase and iodine-starch complex were standardized for enzyme–substrate combinations and process optimization and packed in 2 × 2 cm LDPE bags. The meat samples were packed in polystyrene trays with cling film overwrap and frozen at −18 ± 1 °C. Each sample had a sticker-type TTI attached to the surface of the package. The frozen samples were subjected to temperature abuse at 25 ± 1 °C and 37 ± 1 °C, keeping a control at 4 ± 1 °C. On higher temperature thawing at 25 ± 1 °C, the TTI remained bluish black for 6 h, changed to brown at 12 h, and then changed to yellow at 18 h. At 37 ± 1 °C, color change was not observed in the TTI up to 4 h, then it turned brown at 8 h and to yellow at 12 h. In the control group of frozen meat thawed at 4 ± 1 °C (normal thawing), no color change was observed. From this observation, a color chart was developed to compare TTI color variation with the acceptability of thawed meat. Results have shown that during high temperature thawing of meat, the response of the TTI corroborated well with changes in various meat quality parameters like pH, extract release volume, free amino acids, fluorescein diacetate hydrolysis, total volatile basic nitrogen, D-glucose concentration, shear force, tintometer color units, microbial counts, and sensory scores. It is concluded that a TTI based on the starch-iodine-amylase complex can be successfully used to evaluate the history of high temperature thawing of frozen buffalo meat.

Key words: Time-temperature integrator, buffalo meat, thawing, color, quality

1. Introduction

Accurate monitoring, recording, and controlling of temperature is most important in maintaining the quality and safety of temperature-sensitive food items like meat in the supply chain. It includes postprocessing phases and extends to the time of use by the consumer (1). In most cases, the storage conditions during transportation and at the retail level are out of manufacturer's direct control. Moreover, the product undergoes temperature abuse from the moment that is purchased by the consumer and its intensity depends on the external environmental temperature prevailing in that geographical area, especially important in tropical countries where temperatures above 37 °C are not uncommon. This form of temperature abuse is more relevant with summer months complicated by the phenomenon of global warming, resulting in reduction of the shelf life of the stored product.

In India, buffalo meat contributes 31% of the country's total meat production, and India is the largest exporter of buffalo meat in the world. This meat is mostly exported in a frozen state to distant destinations and the frozen buffalo meat supply chain needs some simple and rapid temperature monitoring tools, which are currently not available in the country.

Temperature is a critical parameter that often deviates from the control specifications and is one of the major factors influencing the rate of microbial development in foods (2), which can ultimately lead to food safety issues. Excessive thawing duration or unusually high temperature thawing may be the most common forms of temperature abuse in the case of meat and meat products. Thus, an efficient system that can monitor any form of temperature abuses like thawing associated with accidental power failure or due to consumer negligence is required to check for food spoilage in the supply chain. This goal can only be achieved by using an appropriate time-temperature integrator (TTI).

TTIs are simple and inexpensive devices that can be attached to the package surface at the unit level, which integrate the cumulative effect of temperature exposure/thawing history of foodstuffs on its quality and safety (3). The response of the TTI is usually clear, continuous, and

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irreversible. TTIs are mainly applied to reflect the time-temperature history of chilled and frozen foods that are temperature-sensitive, such as fresh milk, meat, and sea foods (4,5).

Any fluctuations in temperature during transport and even during final retail display can lead to decreased acceptance of the product or sometimes complete rejection due to severe deterioration in quality. Temperature abuse can also lead to food safety issues by enhancing pathogen growth (6). Therefore, to establish a safety criterion for temperature-abused meat products for human consumption, either direct microbial evaluation or measurement of physicochemical parameters is necessary, but these are normally time-consuming processes. In this context, TTIs can be used as rapid decision-making tools regarding the temperature exposure history and the safety of unduly thawed meat products.

Conventionally, the freshness of meat is assessed chemically on the basis of protein breakdown or fat spoilage, which are suitable spoilage indicators (7). Efficient utilization of on-package indicator sensors for real-time monitoring of buffalo meat quality under refrigerated storage was established by Shukla et al. (8). Microorganisms that can grow and multiply well during high temperature thawing are Enterobacteriaceae, *Brochothrix thermosphacta*, and *Shewanella putrefaciens.* These organisms can produce putrefactive odor compounds and prefer a pH of >6 (9). Among the various physical parameters, extract release volume (ERV) appears to have considerable potential in assessing the spoilage of beef and the same can be applied in buffalo meat to assess spoilage during chiller storage (10). The potential usage of fluorescein diacetate hydrolysis (FDA) assay (A_{490}) to measure microbial load in high temperature thawed buffalo meat was established (11).

Previous studies reported that concentrations of free amino acid, volatile basic nitrogen, and D-glucose in meat can be used as a spoilage indicator, because the sum of free amino acids along with water-soluble protein content increase in meat during storage (12). In another such study, beef stored at 4 °C for 12 days showed a final volatile basic nitrogen concentration of 29.9 mg/100 g compared to 19.2 mg/100 g for beef stored at 0 °C for the same duration. In the same experiment, beef stored at 4 °C with an initial microbial count of log 2.06 cfu/cm^2 showed a final count of log 7.31 cfu/cm^2 (13). Threshold values for various chemical indicators like total volatile basic nitrogen (TVBN), free amino acid, and D-glucose concentration that may coincide with sensorial spoilage of buffalo meat were also reported (14).

Attempts have been made by some researchers to develop TTIs based on different reactions to predict the temperature exposure history of food products. To date, however, there is no research undertaken on the holistic study of TTI response with the quality and safety changes of frozen buffalo meat subjected to higher temperature thawing. Therefore, a research study was undertaken to develop a TTI for rapid assessment of the quality and safety of frozen buffalo meat subjected to thawing at higher temperatures.

2. Materials and methods

2.1. Chemicals

α-Amylase (1,4-α-D-glucan-glucanohydrolase; activity 1300 IU/g) from HiMedia, India; soluble starch extra pure AR from SRL Pvt. Ltd., India; and potassium iodide AR and iodine crystals from Qualigens, Glaxo India Ltd., India, were used for the preparation of TTIs.

2.2. Manufacturing of TTIs

An enzyme–substrate-based TTI using α-amylase and iodine-starch clathrate complex was developed through the following protocol. The substrate was produced by mixing soluble starch and iodine solution. An iodine solution of 0.1 mol/L was prepared by dissolving 1.269 g of iodine crystals and 1.66 g of potassium iodide in 100 mL of double-distilled water. Various combinations of starch and iodine like 1:1, 2:1, and 3:1 were formulated to standardize the optimal substrate suitable for different temperature exposure durations. In order to prepare a powdered form of the substrate, three drying temperatures of 60 °C, 70 °C, and 80 °C were evaluated at a constant duration of 2 h. Different enzyme levels such as 20%, 25%, 30%, 35%, and 40% were then added to the standardized substrate to get the optimum enzyme–substrate combination. The amount of water that must be incorporated into the enzyme–substrate complex for its activation was also standardized (Figure 1).

To select the optimum enzyme concentration, an isothermal storage experiment was conducted. An isothermal storage temperature (37 ± 1 °C) was maintained in an incubator. The temperature of the incubator was strictly monitored by digital probe thermometer (Digi-thermo, WT-2, China). TTIs with different enzyme concentrations such as 20%, 25%, 30%, 35%, and 40% were kept in the incubator for 12 h. The enzyme α-amylase gets activated above 10 °C; therefore, to check the temperature sensitivity of the enzyme, control samples were kept at 4 ± 1 °C for each enzyme concentration. Color response of each enzyme concentration was evaluated at 2-h intervals until the end of enzyme activity. A suitable enzyme level was selected from the above experiment.

The most suitable selected level of enzyme–substrate–water complex formed the basis for the development of the TTI. The above standardized complex mixture was packed in 2 × 2 cm low density polyethylene (LDPE) bags and

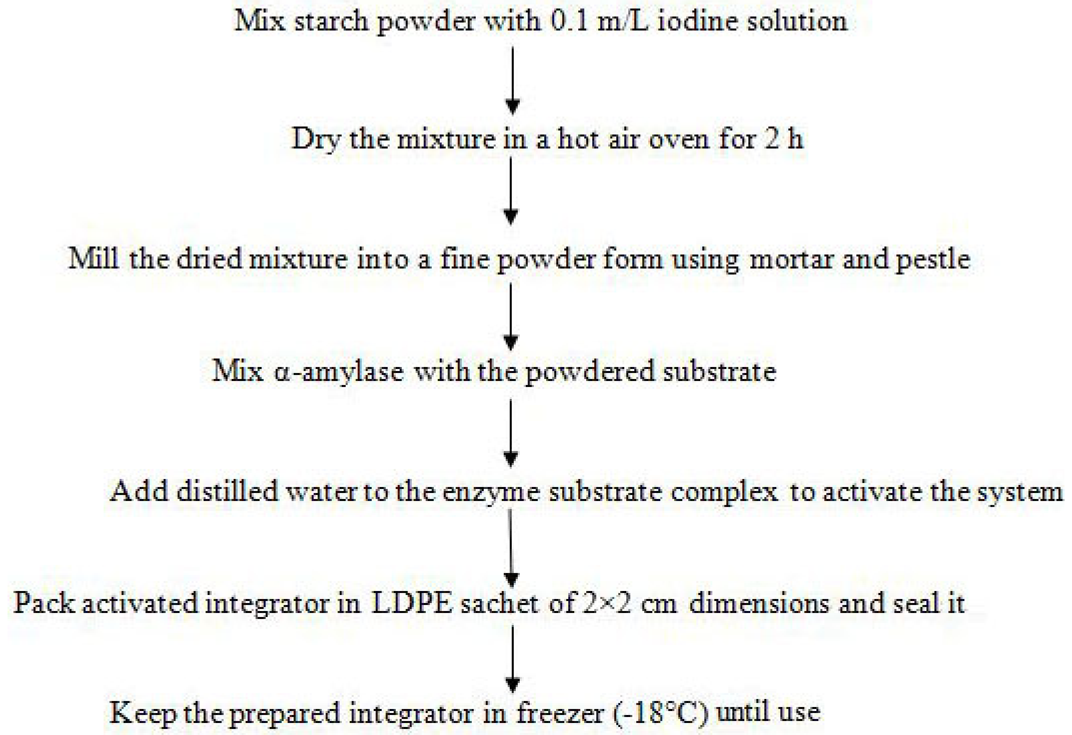

Figure 1. Flow chart for preparation of TTI.

sealed on all sides. This formed the read-to-use TTI, which must be frozen if not intended for immediate use in order to avoid the activation of the enzyme. This sticker-type TTI was used on the surface of the package for monitoring the quality of the packaged meat.

2.3. Meat sample preparation

The meat samples were collected from a buffalo slaughter house, Bareilly district, Uttar Pradesh State, India. The thigh muscles without excessive fat and connective tissues were collected from nine different buffaloes, which were slaughtered according to traditional halal method and brought to the lab within 4 h of slaughter. Meat collected from nine different carcasses was packed separately in LDPE bags and kept in a chiller room maintained at 7–10 °C for 24 h for the rigor mortis to complete so as to avoid cold shortening and excessive drip loss. After the initial chilling period, the total meat was divided into 3 replicates and each replicate represented meat from three randomly chosen carcasses. Each replicate of meat was divided into 7 experimental groups (C, T_1 to T_6) and packed in polystyrene foam trays covered with polyvinyl chloride cling film. A ready-to-use TTI was attached over the cling film's outer surface and all the samples were stored in a deep freezer at –18 ± 1 °C (Vest Frost, Denmark). These groups of frozen meat were used further for high temperature thawing experiments.

2.4. Experimental design for higher temperature thawing

Temperature-controlled incubators were used for the higher temperature thawing experiment, in which incubator 1 was maintained at 25 ± 1 °C and incubator 2 at 37 ± 1 °C. Frozen buffalo meat with TTIs were subjected to different thawing conditions as mentioned below:

C- Control: Frozen meat kept at regular thawing temperature (4 ± 1 °C).

T_1, T_2, T_3: Frozen meat kept at higher thawing temperature of 25 ± 1 °C (incubator 1) for 6 h, 12 h, and 18 h, respectively,

T_4, T_5, T_6: Frozen meat kept at higher thawing temperature of 37 ± 1 °C (incubator 2) for 4 h, 8 h, and 12 h, respectively.

During different thawing conditions, color change in the TTI attached to each group was compared against the control group. Meanwhile, samples for each exposure interval were analyzed for various meat quality parameters. Each experimental analysis was performed in duplicates in all three replicates (n = 3).

2.5. Meat quality evaluation

2.5.1. pH and extract release volume (ERV)

The tissue homogenate was prepared by blending 10 g of meat sample with 90 mL of distilled water using an Ultra Turrax tissue homogenizer (Ultra Turrax IKA, Model T18 Basic, IKA Wares Inc., Wilmington, USA) for 1

min. The pH was recorded by immersing the combined glass electrode of the digital pH meter (Model CP 901, Century Instrument Ltd., Chandigarh, India) into the meat homogenate. The ERV was estimated according to the procedure described by Strange et al. (15) with suitable modifications.

2.5.2. Fluorescein diacetate (FDA) hydrolysis

The FDA hydrolysis of meat samples was measured according to the procedure described by Venkitanarayanan et al. (16) with suitable modifications. The FDA hydrolysis values were expressed as mean absorbance at 490 nm using a spectrophotometer (Model DU 640, Beckman, USA).

2.5.3. Free amino acid (FAA) content

The FAA concentration was determined in accordance with the procedure described by Rosen (17) following the colorimetric ninhydrin method with suitable modifications. The α-amino acid present in the meat was estimated as ninhydrin reactive substance. The free amino acid content of meat was expressed as mg per 100 g of meat.

2.5.4. Total volatile basic nitrogen (TVBN) estimation

TVBN content of buffalo meat samples were determined by the procedure of Pearson (18) following a microdiffusion technique with slight modifications. TVBN content was calculated by using the following equation:

TVBN (mg/100 g of meat) = Reading of burette (volume of 0.02 N H_2SO_4 consumed) × normality of acid used for titration × 14 × 100.

2.5.5. D-glucose estimation

D-glucose concentrations in different meat samples were determined using a glucose oxidase/peroxidase assay kit (Sigma-Aldrich, USA). The optical density of the reaction mixture was determined with a spectrophotometer (Model DU 640, Beckman, USA) at 540 nm and converted to mg D-glucose per mL of aliquot using a glucose standard curve. The D-glucose content of meat was expressed as mg per 100 g of meat.

2.5.6. Lovibond tintometer color value and Warner–Bratzler shear force value

Lovibond tintometer red (LTCU ‘R’) and yellow (LTCU ‘Y’) color units were recorded using a Lovibond Tintometer (model F, Greenwich, UK). The hue angle and chroma (saturation index) values of meat were determined by using the formula $\tan_{-1}^{(b/a)}$ and $(a^2 + b^2)^{1/2}$, respectively , where a = red unit and b = yellow unit. The shear force value of the samples was recorded as per the method of Berry and Stiffler (19) with suitable modifications using a Warner–Bratzler shear press (Model: 81031307, G. R. Elect Mfg. Co., USA) and results were expressed as kg/cm^2 .

2.5.7. Microbiological analysis

All the microbiological parameters of meat samples including total plate count, yeast and mold count, and psychrophilic counts were determined as per the methods described by the APHA (20). Duplicate plates were prepared and the counts were expressed as colony-forming units (cfu) per gram.

2.5.8. Sensory analysis

The sensory qualities of control as well as treatment samples were judged on the basis of appearance, color, odor, and sliminess as suggested by the AMSA (21). The samples were subjected to sensory evaluation by trained panelists (n = 7) consisting of scientists and postgraduate students of the Division of Livestock Products Technology, Indian Veterinary Research Institute, Izatnagar.

2.5.9. Statistical analysis

A randomized block design with three completely random replicates was used for experiments and the data generated for different meat quality parameters were compiled and analyzed using SPSS 20.0 for Windows (IBM Corp., Armonk, NY, USA). The smallest difference ($D_{5\%}$) for two means was reported as significantly different ($P < 0.05$).

3. Results

3.1. Effect of substrate combination, drying temperature, and enzyme level on TTI response

Various substrate combinations, drying temperatures, and enzyme concentrations were standardized for the preparation of the ready-to-use TTI. At any constant enzyme concentration, the substrate combination was found to be critical in determining the speed of color changing reaction. A 2:1 combination of starch and iodine solution was selected for TTI preparation since it showed a moderate reaction speed when mixed with the enzyme and exposed to higher temperature. The temperature of drying was standardized as 60 °C based on the easiness of powdering of the dried substrate and fineness of the powder obtained.

3.2. Comparison of the TTI color response with meat quality changes

All the treatment and control groups were compared for changes in various meat quality parameters along with TTI color response after higher temperature thawing. All the meat quality parameters were found to be affected when the frozen buffalo meat was thawed at higher temperature. The effects of higher temperature thawing on meat quality parameters were characterized using the response of the TTI that was attached to each treatment group (Figures 2 and 3).

Treatment groups T_3 and T_6 showed that the meat was not acceptable immediately after the thawing period based on sensory evaluation. It was observed that the TTI attached to both T_3 and T_6 showed a complete color change from its initial bluish black color (original TTI color) to

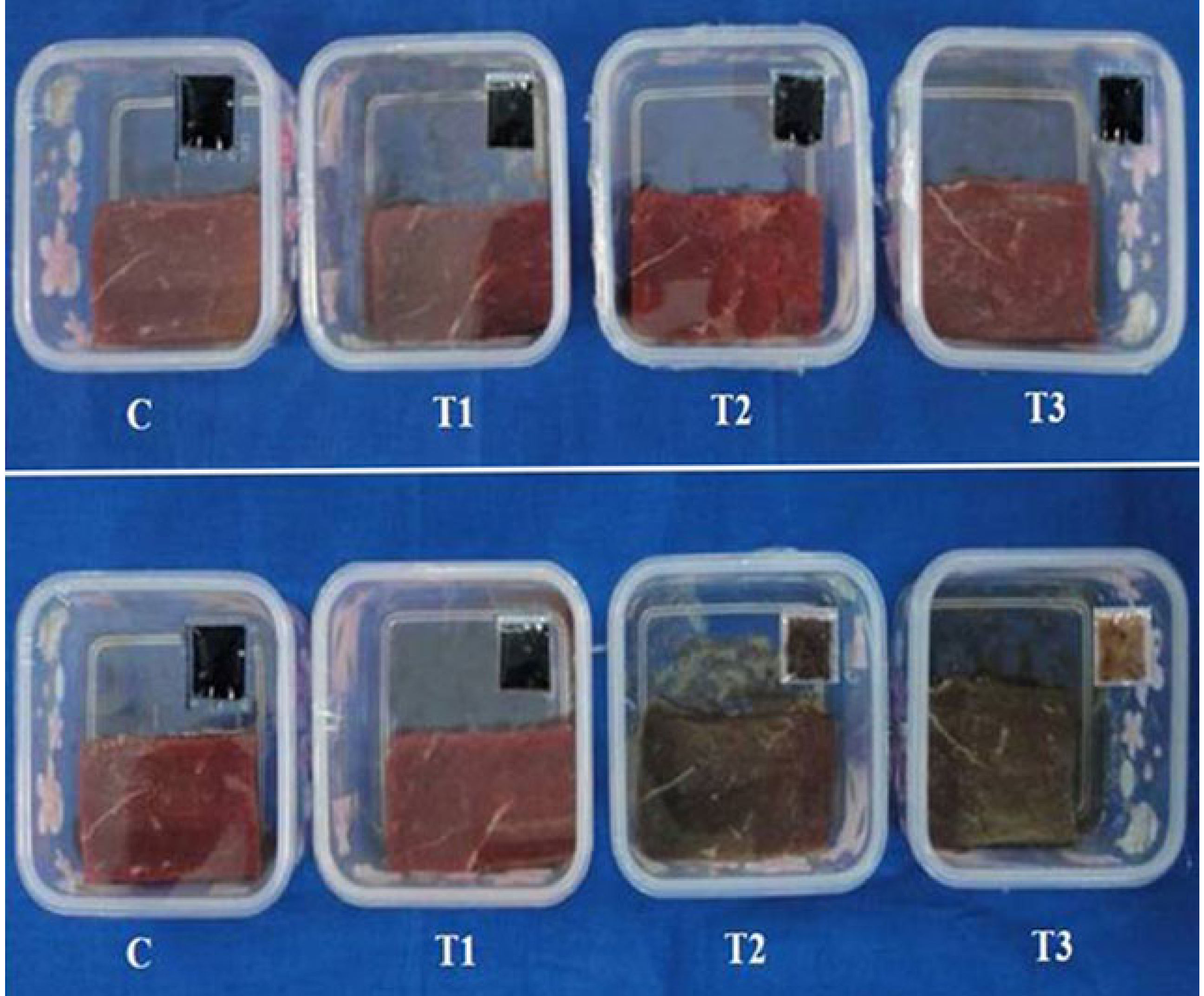

Figure 2. Control and treatment samples groups before (above) and after (below) higher temperature thawing at 25 ± 1 °C (C = control; T_1 = treatment 1 (thawed at 25 ± 1 °C for 6 h); T_2 = treatment 2 (thawed at 25 ± 1 °C for 12 h); T_3 = treatment 3 (thawed at 25 ± 1 °C for 18 h).

a light yellow color (full change in TTI color). However, some of the physicochemical parameters were well within the limits for these samples. The meat quality changes were found to be well correlated with the color changes observed in the TTI attached to each treatments group after higher temperature thawing. T_1 and T_4 showed the least changes and T_2 and T_5 showed moderate changes in meat quality parameters. This can be clearly established from the no color change response of the TTIs attached to T_1 and T_4 and the moderate color change (brown color) of the TTIs attached to T_2 and T_5. Therefore, it was concluded that the thawing of frozen buffalo meat at 37 ± 1 °C for 12 h or at 25 ± 1 °C for 18 h continuously or intermittently causes detrimental effects on meat quality and such changes can be monitored very easily by observing the TTI color response. A color response chart was developed for easy comparison of TTI color change with changes in meat quality parameters of higher temperature thawed frozen buffalo meat (Figure 4).

3.3. Physicochemical parameters

Temperature-abused buffalo meat samples showed a significantly ($P < 0.05$) higher pH, FDA hydrolysis, TVBN content, and FAA content than control samples immediately after the exposure period, but the ERV and D-glucose concentration showed a significantly ($P < 0.05$) lower value in temperature-abused buffalo meat samples compared to control samples after the exposure period (Table 1).

Figure 3. Control and treatment samples groups before (above) and after (below) higher temperature thawing at 37 ± 1 °C (C = control; T_4 = treatment 4 (thawed at 37 ± 1 °C for 4 h); T_5 = treatment 5 (thawed at 37 ± 1 °C for 8 h); T_6 = treatment 6 (thawed at 37 ± 1 °C for 12 h).

Figure 4. Color chart developed for comparing TTI color response with meat quality after higher temperature thawing.

3.4. Lovibond tintometer color units and Warner–Bratzler shear force value

The temperature-abused buffalo meat samples showed significantly (P < 0.05) lower Lovibond tintometer red and yellow units, chroma values, and Warner–Bratzler shear force values after temperature abuse as compared to control sample. However, the hue angle exhibited inconsistent values between treatment samples and control samples after the temperature abuse period (Table 1).

3.5. Microbiological and sensory parameters

Total plate count, psychrophilic count, yeast and mold count, and *Pseudomonas* count of temperature-abused buffalo meat samples showed significantly (P < 0.05) higher values than control samples after exposure (Table 2). Temperature-abused buffalo meat samples showed a significantly (P < 0.05) lower appearance score, color score, odor score, and sliminess score compared to control samples after the exposure period (Table 2).

4. Discussion

4.1. Standardization of the TTI for optimum color response

The bluish black color of the substrate was due to a clathrate compound produced between starch and iodine. This color was directly proportional to the degree of polymerization of starch (22). The enzyme used in the present TTI hydrolyzes this complex to produce a light yellow end product when exposed to higher temperature for sufficient duration, so there should be equilibrium between the number of clathrate complexes formed and the number of enzyme molecules used in the TTI. Different levels of enzyme in the TTI were evaluated by exposing the TTI to a constant temperature (37 ± 1 °C) until the end of the reaction and different durations were noticed with different enzyme concentrations. A 40% level of enzyme on whole TTI weight was standardized for the final TTI preparation to evaluate high temperature thawing of frozen meat. The developed TTI with 40% enzyme level did not show any visible color change for up to 6 h of storage at 37 ± 1 °C. A moderate change in TTI color was noticed after 8 h of exposure at 37 ± 1 °C. After 10 h of exposure at 37 ± 1 °C, the color change of the TTI was more than that at 8 h of exposure. A complete color change to a final light yellow color was noticed in the developed TTI after 12 h of exposure at 37 ± 1 °C (Figure 5)

4.2. Various meat quality parameters upon abusive thawing

In buffalo meat product quality studies reported by researchers, meat keema prepared from young buffalos showed a mean pH value of 6.13 ± 0.02 (23) and buffalo meat curry showed a pH value of 5.90 ± 0.03 (24) after 72 h of storage at an ambient temperature of 37 ± 1 °C. These studies agreed with the present finding that the pH of buffalo meat increased with increased duration of thawing

Table 1. Physicochemical properties and instrumental analysis values of control and treatment samples immediately after temperature exposure study (mean ± SE).

Parameters	Treatments						
	C	T_1	T_2	T_3	T_4	T_5	T_6
pH	5.27 ± 0.01^c	5.28 ± 0.01^c	5.29 ± 0.02^c	5.31 ± 0.02^c	5.29 ± 0.01^c	5.45 ± 0.01^b	5.54 ± 0.01^a
ERV (mL)	29.00 ± 0.77^a	24.58 ± 0.24^b	23.58 ± 0.20^b	21.18 ± 0.46^c	23.73 ± 0.31^b	23.25 ± 0.71^b	20.98 ± 0.2^c
FDA hydrolysis	0.24 ± 0.02^f	0.27 ± 0.02^d	0.40 ± 0.02^c	0.48 ± 0.02^a	0.26 ± 0.03^e	0.42 ± 0.01^b	0.48 ± 0.02^a
FAA (mg/100 g)	4.80 ± 0.07^g	5.30 ± 0.04^f	8.68 ± 0.04^d	18.83 ± 0.21^a	6.57 ± 0.14^e	9.48 ± 0.19^c	16.27 ± 0.13^b
TVBN (mg/100 g)	8.13 ± 0.09^f	8.70 ± 0.04^e	10.72 ± 0.04^d	15.63 ± .09^a	9.03 ± 0.12^e	12.02 ± 0.27^c	14.77 ± 0.10^b
D-glucose (mg/100 g)	142.00 ± 1.03^a	123.25 ± 0.91^c	115.00 ± 0.98^e	95.13 ± 0.81^f	135.30 ± 0.88^b	118.41 ± 0.69^d	95.69 ± 0.79^f
WBSF value (kg/cm^2)	7.27 ± 0.08^a	7.10 ± 0.08^{ab}	6.08 ± 0.08^c	5.07 ± 0.07^d	6.95 ± 0.07^b	6.05 ± 0.06^c	5.02 ± 0.07^d
Redness value	6.40 ± 0.04^a	5.00 ± 0.50^c	3.93 ± 0.03^d	3.62 ± 0.04^e	5.32 ± 0.03^b	3.90 ± 0.05^d	3.53 ± 0.03^e
Yellowness value	4.57 ± 0.02^a	2.83 ± 0.00	2.70 ± 0.04^{cd}	2.64 ± 0.05^d	2.78 ± 0.05^{bc}	2.67 ± 0.06^{cd}	2.40 ± 0.05^e
Chroma	7.86 ± 0.03^a	5.66 ± 0.03^c	4.77 ± 0.03^d	4.60 ± 0.05^e	6.00 ± 0.05^b	4.58 ± 0.05^e	4.43 ± 0.05^f
Hue angle	35.50 ± 0.21^b	27.83 ± 0.51^d	34.55 ± 0.59^b	38.11 ± 0.55^a	27.69 ± 0.34^d	31.70 ± 0.46^c	36.96 ± 0.66^a

C = Control; T1 = treatment 1 (exposed at 25 ± 1 °C for 6 h); T_2 = treatment 2 (exposed at 25 ± 1 °C for 12 h); T_3 = treatment 3 (exposed at 25 ± 1 °C for 18 h); T_4 = treatment 4 (exposed at 37 ± 1 °C for 4 h); T_5 = treatment 5 (exposed at 37 ± 1 °C for 8 h); T_6 = treatment 6 (exposed at 37 ± 1 °C for 12 h). Means with different superscripts in the same row indicate significance (P < 0.05).

Table 2. Microbiological and sensory properties of control and treatment samples immediately after temperature exposure study (mean ± SE).

Parameters	Treatments						
	C	T_1	T_2	T_3	T_4	T_5	T_6
TPC (log cfu/g)	3.44 ± 0.03^e	4.21 ± 0.02^d	5.61 ± 0.03^c	6.20 ± 0.01^b	4.29 ± 0.04^d	6.24 ± 0.02^b	6.86 ± 0.02^a
PSY (log cfu/g)	3.56 ± 0.04^e	3.60 ± 0.02^e	3.51 ± 0.03^e	5.21 ± 0.00^c	4.03 ± 0.06^d	5.47 ± 0.06^b	5.71 ± 0.03^a
YMC (log cfu/g)	3.07 ± 0.03^f	3.57 ± 0.05^d	3.60 ± 0.01^d	5.05 ± 0.02^b	3.37 ± 0.06^e	4.58 ± 0.05^c	5.57 ± 0.02^a
PSD (log cfu/g)	3.33 ± 0.03^d	3.42 ± 0.05^d	4.53 ± 0.02^c	5.26 ± 0.06^b	4.58 ± 0.05^c	5.30 ± 0.07^b	5.81 ± 0.04^a
Appearance	5.00 ± 00^a	4.05 ± 0.04^{bc}	3.93 ± 0.05^c	2.23 ± 0.05^e	4.12 ± 0.04^b	3.56 ± 0.06^d	1.85 ± 0.05^f
Color	5.00 ± 00^a	3.33 ± 0.06^c	3.19 ± 0.05^c	2.14 ± 0.05^d	3.83 ± 0.05^b	3.18 ± 0.05^c	1.94 ± 0.04^e
Odor	5.00 ± 00^a	4.50 ± 0.08^b	3.69 ± 0.07^c	2.14 ± 0.06^e	4.50 ± 0.08^b	3.14 ± 0.09^d	1.86 ± 0.04^f
Sliminess	5.00 ± 00^a	4.38 ± 0.06^b	3.18 ± 0.05^d	2.64 ± 0.07^f	3.93 ± 0.04^c	3.12 ± 0.08^d	2.82 ± 0.07

C = Control; T1 = treatment 1 (exposed at 25 ± 1 °C for 6 h); T_2 = treatment 2 (exposed at 25 ± 1 °C for 12 h); T_3 = treatment 3 (exposed at 25 ± 1 °C for 18 h); T_4 = treatment 4 (exposed at 37 ± 1 °C for 4 h); T_5 = treatment 5 (exposed at 37 ± 1 °C for 8 h); T_6 = treatment 6 (exposed at 37 ± 1 °C for 12 h). Means with different superscripts in the same row indicate significance (P < 0.05). TPC: total plate count; PSY: psychrophilic count; YMC: yeast and mold count; PSD: Pseudomonas count

at higher temperature. In the context of decrease in ERV, the present finding was in agreement with the study of Shelef and Jay (25). These authors reported a negative correlation between ERV values and microbial counts of beef, and a similar observation was noted in the present study, too. Moreover, a gradual decrease in the ERV value of buffalo meat in chiller storage was also reported (10). The present study established a minimum FDA hydrolysis of 0.48 ± 0.02 (absorbance, mean ± SE), which coincided with a rejectable total microbial count in buffalo meat. In one such similar study conducted by Venkitanarayanan et al. (16) on biceps femoris steaks under thawing and temperature abuse conditions at 25 °C, they reported a FDA hydrolysis value of 0.5 ± 0.02 when the bacterial load was log 6.8 cfu/cm^2.

The significant (P < 0.05) increase in FAA content of treatment samples was due to increased breakdown of meat proteins by bacterial proteases. This was supported by the finding that meat with higher *Pseudomonas* count always produces a higher level of free amino acids (26). The TVBN content of severely thawed buffalo meat nearly reaches the upper limit of sensorial rejection, which is 16.5 mg of volatile nitrogen per 100 g of beef (27). The significant decrease (P < 0.05) in D-glucose concentration recorded for treatment and control samples in the present study was due to the growth of glucose utilizing bacterial microflora. Low concentration of D-glucose in spoiled samples suggested the possible involvement of pseudomonads, since fresh meat spoilage under aerobic conditions was usually initiated by exhausting the available glucose in meat (26).

The significant decrease (P < 0.05) in instrumental color scores like redness, yellowness, and chroma for treatment samples was due to the effect of higher temperature exposure on the major meat pigment myoglobin. The autoxidation rate of myoglobin to metmyoglobin increased at high temperature and metmyoglobin imparted a brown color to the samples subjected to high temperature thawing. This is also supported by the finding that, as the storage period increased, the metmyoglobin accumulation in meat tissue increased consistently (28). In the case of Warner–Bratzler shear force, the decreased trend associated with longer thawing was supported by the findings of French et al. (29) in steer meat.

Nychas (9) reported that *P. fragi* and *P. putida* are the important spoilage species in minced beef. Bruckner et al. (30) established the importance of storage temperature in attaining the microbial shelf life in fresh meat. They demonstrated a time gap of 165.8 h at 2 °C compared to 45.5 h at 15 °C to reach an unacceptably higher *Pseudomonas* count (log 7.5 cfu/g) and sensory spoilage characteristics in fresh loin.

Since the color of meat decreased significantly after temperature abuse, the appearance score was also reduced. Attributes like sliminess, loss of moisture, and protein denaturation have also contributed to a decreased appearance score in temperature-abused meat samples. From the present study it was observed that the off odor that develops due to surface microbial contamination is one of the major causes of spoilage in meat. Nychas et al. (12) showed the first signs of spoilage in meat to be caused by the formation of fruity, sweet-smelling esters, followed

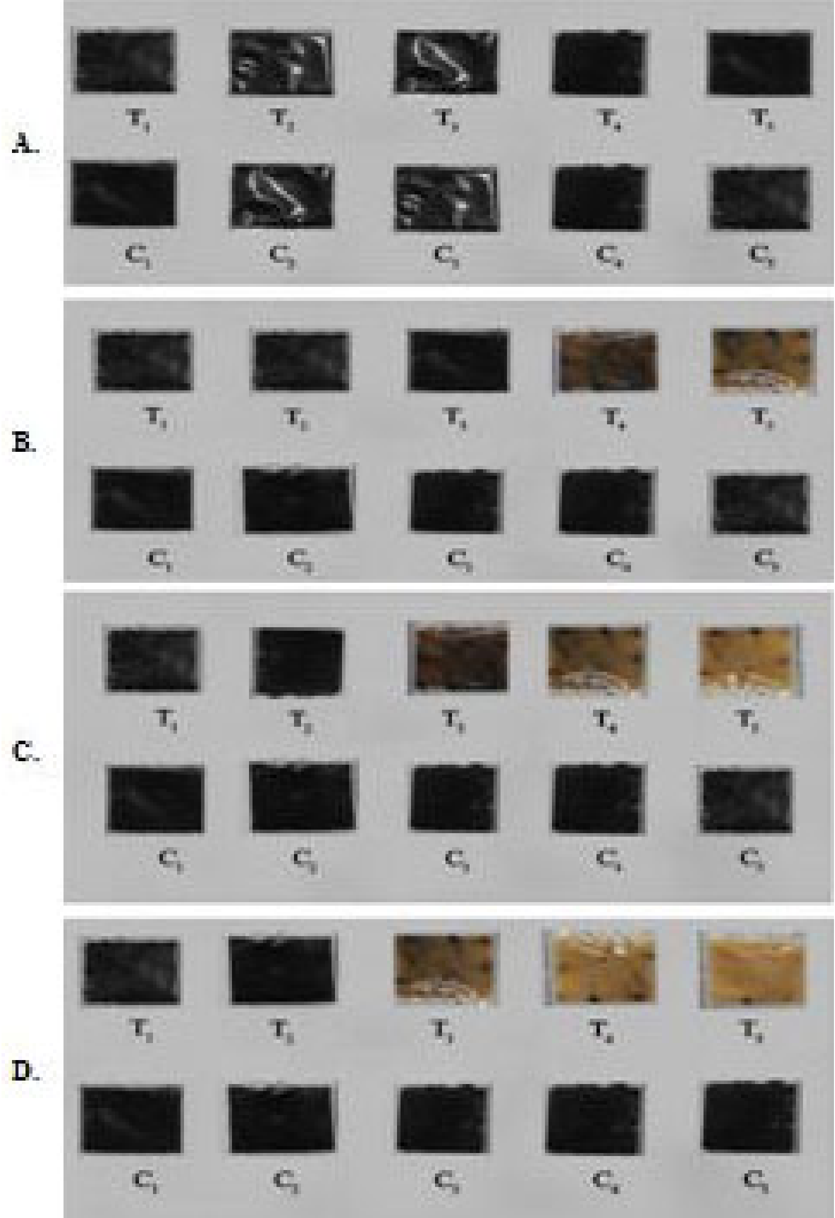

Figure 5. Initial color of TTI (A). Color change in TTI during isothermal storage at 37 ± 1 °C for 8 h (B), 10 h (C), and 12 h (D). T_1 to T_5: ready to use TTIs with increasing enzyme concentrations; C_1 to C_5: control TTIs with increasing enzyme concentrations.

by the formation of putrid sulfur compounds. Nychas et al. (9) also correlated the sliminess development in meat with the growth of bacteria mainly belong to *Pseudomonas*, *Lactobacillus*, and *Enterococcus*.

4.3. Conclusions

An enzyme–substrate-based TTI that is activated above 10 °C and produces a visible color change from bluish black to light yellow was developed. The color change in the TTI and quality changes in buffalo meat were found to be correlated well after higher temperature thawing. A complete color change in the TTI was found to coincide with the development of unacceptable quality characteristics in higher temperature thawed frozen buffalo meat. The importance of this developed TTI as a decision-making tool for consumers was established by the present research and further research to modify the TTI, which can be suitably used for different meat food products, is recommended.

Acknowledgment

The authors are thankful to the Director and Joint Director (Research) of the Indian Veterinary Research Institute, Izatnagar, Barielly, India, for the facilities provided to conduct the research.

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