Applications of Time–Temperature Integrator (TTI) as a Quality Indicator of Grounded Pork Patty

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Abstract

Time-temperature integrators (TTIs) are simple and cost-efficient tools which may be used to predict food quality. Enzymatic TTIs are devised to indicate food quality in the form of color alterations from green to red, based on the cumulative impacts of temperature and time period on the enzymatic reactions. In this study, the quality of ground beef patties was investigated for the parameters of pH levels, color, VBN, water holding capacity, and total microbial counts, depending on various storage temperatures (5, 15, and 25°C). TTIs were attached to the surface of the ground beef patties in order to evaluate the degree of correlating colorimetric changes with the determined quality parameters. Through the Arrhenius equation, activation energy and constant reaction rates of TTI, VBN, and total microbial counts were calculated as to observe the relationship between enzymatic reactions of the TTI and food spoilage reactions of the ground beef patties. VBN and total microbial counts were already increased to reach decomposition index (VBN: 20, total microbial count: 7-8 Log CFU/g) of meat at middle stage of storage period for each storage temperature. Although activation energy of TTI enzymatic reactions and food spoilage reactions of the ground beef patties were similar, the change of TTI color was not a coincidence for food spoilage at 5°C and 15°C of storage temperature. It was suggested that TTI should be designed individually for storage temperature, time, type of meat, or decomposition index of meat.

Key words: time-temperature integrator, beef patty, storage, quality indicator

Introduction

Throughout history, food products and services that promote personal well-being have been sought. Consumers have demanded quality factors of food especially in the meat product industry as consumption of meat is gradually increased. Hence, it is sought to control the quality of meat at every stage of production, such as the rearing of the animals, slaughter, distribution and storage. Quality control is helpful both to the consumer and to the manufacturer, because consumer is assured a high quality meat product and the manufacturer can reduce economic loss by decreasing unexpected spoilage of meat products (Bruckner *et al.*, 2013; Taoukis *et al.*, 1999; Vaikousi *et*

al., 2009).

Temperature-sensitive products are carried through the cold-chain and are used to check the history of storage and delivery conditions until the point of consumption. Many food processing enterprises have applied Hazard Analysis Critical Control Point system (HACCP) process to keep up with customer needs. However, it is impractical to test product qualities such as chemical, physical, or microbial properties before purchasing in the market. Consumers can merely confirm that the designated expiry date is appropriate and personally observe for color, ion sealed packages, and malodour, when available. (Byeon *et al.*, 2009; Shin *et al.*, 2006). Moreover, thermal processing has been well-known as an effective way of preventing or eliminating photogenic bacteria or viruses from food products such as meat, fish, and poultry products, but this type of processing is one of the last control points in HACCP (Orta-Ramirez and Smith 2002; Van Loey *et al.*, 1996). Obviously, uncooked meat products are

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exposed to various environmental stresses such as temperature, oxygen, light, and so on, before cooking.

Forecasting systems for food quality have been developed over past decades. Time-temperature integrators (TTIs) are used to predict food quality and it has been considered that it is essential tool in food groups. TTIs are devised to show food quality as a color alteration which occurs at varying rates due to exposure to varying temperature. Moreover, TTIs have low-cost and high-efficiency. (Ellouze *et al.*, 2011; Kim *et al.*, 2012b). Three types of TTI have been developed, which are based on diffusion, enzyme, and polymer sensors, which are classified by reaction material or method. The reaction can be a physical, chemical or enzymatic irreversible change (Park *et al.*, 2009; Vaikousi *et al.*, 2009). Vitsab® Smart Labels TTIs, which are highly efficient and simple, were used in this study. This TTI is based on enzymatic reaction and uses lipase. The enzyme induces lipolysis which affects a decrease of pH that causes a color-change of the indicator from green to red (Kim *et al.*, 2012a; Smolander *et al.*, 2004). Finally we aim to recognize the state of product quality through the color of the TTI. There are many instances of applications of TTI within the food industry (Bobelyn *et al.*, 2006; Claeys *et al.*, 2002; Giannakourou and Taoukis 2002; Shin *et al.*, 2006; Taoukis *et al.*, 1999; Vaikousi *et al.*, 2009).

Even if the application of TTIs on food products has increased because of their cost-effectiveness, simplicity, and ease-of-use, they still must be calibrated to the chemical, physical, microbial or sensory properties of the products to which they are applied (pH, color, VBN, TBA, water holding capacity, total microbial count, sensory evaluation etc.) depending on the storage period and processing method (Jeong *et al.*, 2006; Jin *et al.*, 2002; Kim *et al.*, 2012b; Shin *et al.*, 2006). These methods of determination take time, cost and skilled technicians to observe the freshness of meat products, and therefore, it is of great advantage to carry out a detailed investigation of the relationship between TTI and quality of meat products, using the TTI thereafter. In this study, the objective was to investigate correlation between TTI and ground beef patty quality. Therefore, we observed changes of TTI's color and meat quality such as meat color, pH, VBN, water-holding capacity, and total microbial count, as measured during storage under variable temperature conditions. Arrhenius activation energy was calculated to represent the correlation between TTI and GBP quality at the same temperature and period.

Materials and Methods

Materials

Time-temperature integrators (TTI, Vitsab® Smart Labels, Sweden) which are an enzymatic reaction type TTI were donated by Vitsab International. Ground Beef was obtained from a meat grocery in the Hwayang Market. Potassium carbonate and Brunswick solution was purchased from SAMCHUN Chemical Co. (Korea). Sulfuric acid was provided by PFP (PFP Matunoen chemicals Ltd, Japan) and sodium hydroxide was bought from SHOWA (SHOWA, Japan). For the microbial test, plate count agar (PCA) was obtained from Difco (Becton Dickinson and Company, USA).

Sample preparation

Seventy grams of ground beef was formed using a petri dish (Internal dimension: 85.6 mm × 12.6 mm, SPL, Korea) to result in a uniform and reproducible sample shape and volume. TTIs were attached to the middle of the surface of the ground beef patties (GBPs). GBPs with attached TTI were then stored at 5°C, 15°C, or 25°C for predetermined storage periods. Change in GBPs quality and TTI was observed at certain periods in triplicate. Observations of GBPs quality ceased when the a^* -value of TTIs (1st condition) was over 20-25, or when VBN (2nd condition) was greater than 20, which was based on the findings of a previous research (Chun *et al.*, 2009).

Color

CIE Lab color values were measured from the surface of GBPs with a color reader (CR-10, Konica Minolta Sensing Inc., Japan) which was calibrated using a standard white plate. The CIE L^* , a^* , and b^* values were designated as indicators of lightness, redness, and yellowness, respectively. The samples were determined and three measurements were taken from the surface of each sample. a^* -values were then considered as quality attribute depending on storage temperature. The change of a^* -value was calculated over time to evaluate the constant reaction rate and activation energy, using the Arrhenius equation.

Volatile basic nitrogen (VBN)

VBN of samples was measured by Conway's Micro diffusion method of Korean Food Standards Codex (KFDA, 2012). Three Conway's tools were cleaned with a neutral detergent to remove any containment. To the edge of the outer ring of each unit was applied sealing agent. Five

grams of GBP from a homogenate was mixed with 25 mL distilled water and was then filtered. The 1 mL of filtrate was put into the lower part of outer ring and 1 mL of saturated K_2CO_3 solution was carefully pipetted into the upper part of outer ring. One mL of 0.01 N H_2SO_4 was added to the inner ring of the unit and immediately the units were covered and closed with clip. Solution of outer ring was mixed carefully to prevent any entering the inner ring. The Conway diffusion cell was filled with the filtrate of GBPs and was kept at 25°C for 1 h. One drop of Brunswick solution was added to the inner ring of the unit and the filtrate of GBPs was then titrated with 0.01 N NaOH solution. The VBN value was calculated as

$$VBN(mg\%) = 0.14 \times \frac{(b-a) \times f}{W} \times 100 \times d$$

where a was amount (mL) of 0.01 N NaOH solution for filtrate of GBPs, b was amount (mL) of 0.01 N NaOH solution for control, d was dilution factor, W was GBPs amount (mg) and f was titer of 0.01 N NaOH solution.

pH

Five grams of GBP was mixed with 20 mL of water and was homogenized at 10,000 rpm for 3 min using SMT process homogenizer (SMT Co. Ltd., Japan). The pH of GBPs was measured in triplicate using a pH meter (pH 900, Precisa Co., Swiss).

Water holding capacity

Water holding capacity (WHC) of GBPs was determined (Hong *et al.*, 2008). One gram of GBP was weighed and placed in a centrifuge tube along with gauze as an absorbent. The samples were centrifuged for 10 min at 3,000 rpm and 4°C in a refrigerated centrifuge (RC-3, SORVALL Co., USA). After centrifuging, the meat was removed from the tube and the weights of the centrifuge tubes before and after drying were determined. WHC was expressed as the percentage of moisture remaining in GBP samples. All measurements were carried out in triplicate.

Total microbial count

The total microbial count of GBPs stored at 5°C, 15°C, and 25°C was determined at certain periods. One gram of sample was diluted in 9 mL pre-sterilized 0.85% NaCl solution. One milliliter of bacteria solution was inoculated on the surface of a PCA plate. The plates were incubated at 37°C in incubator for 24-48 h. Determination of CFU/g was then carried out.

Correlation of TTI, VBN, or total microbial count by Arrhenius equation

Specially, the a^* -value of TTI, VBN and total microbial count of GBPs kept at various storage temperatures was calculated by Arrhenius equation. The Arrhenius equation defines the quantitative basis of the relationship between the activation energy (E_a) and constant reaction rate (k) as

$$k = A \exp\left(-\frac{E_a}{RT}\right)$$

where R is the universal gas constant (8.314 J/mol·K) and T is absolute temperature (K).

The E_a of TTI, VBN, or total microbial count depending on various storage temperatures was calculated and plotted against the reciprocal of absolute temperature according to Eq. 1. The activation energy was then calculated from the logarithmic form of the Arrhenius equation. The parameter E_a was calculated as a slope by plotting $\ln k$ as a function of $1/T$ according to the following equation.

$$\ln k = \ln A - \frac{E_a}{R} \frac{1}{T}$$

Statistical analysis

The data were analyzed by using one-way analysis of variance with storage periods. An analysis of variance was performed on all the variables using the General Linear Model (GLM) procedure (SAS 9.3, SAS Institute, USA). Differences among the means were compared using Tukey's Studentized Range (HSD) Test ($p < 0.05$).

Results and Discussion

Color

As described at sample preparation, the a^* -value of the TTI or VBN were investigated as representative factors to indicate food spoilage in previous study. Through the previous study, all observation of GBPs' characteristics such as color, VBN, pH, water holding capacity, and microbial test was finished when the a^* -value of TTI was 20-25. Fig. 1 shows that changes in the a^* -value of both TTI and GBPs occurred during storage periods. TTI was kept at 25°C, 15°C, or 5°C and color observation were finished after 24 h, 196 h and 288 h respectively according to the a^* -value (20-25). The total storage period was also subdivided into initial stage, middle stage, and final stage, because each sample temperature had different a storage

Fig. 1. a^* -value of TTI or meat as storage temperature (A: 25°C, B: 15°C, C: 5°C). Vertical bars indicate standard deviations (n=3).

period (Table 1). The a^* -value of TTI only was significantly increased by raising the storage temperature at 25°C and 15°C ($p < 0.05$), however, it was no difference among samples at 5°C storage temperature until VBN was over 20. There was no remarkable change in the a^* -value of GBPs kept at 15°C or 5°C. The b^* -value of TTIs (Fig. 2) fluctuated during the storage period and the b^* -value of GBPs was quite stable at around 10 to 14 ($p > 0.05$). The L^* -value was not changed during storage periods (results not shown). Fig. 3 shows the real color of TTI and GBPs. TTI was changed green-yellow-red and GBPs were changed from blood red-light brown-deep blood red, in order of storage period, except for 5°C storage temperature. The

a^* -value was the most changeable factor to indicate and predict the state of food quality, and moreover, consumers may easily recognize this TTI color change (Byeon *et al.*, 2009; Chun *et al.*, 2009; Kim *et al.*, 2012a).

Volatile basic nitrogen (VBN)

The VBN value of GBPs depended on storage temperature (5°C, 15°C, and 25°C). VBN value was increased with storage period. It was quickly and steadily increased up to 22 mg% at 21 h with 25°C of storage temperature (Fig. 4A). Interestingly, VBN was dramatically increased at 75 h at 15°C and 5°C of storage temperature (Fig. 4B). The a^* -value was very low at the same time for both storage temperatures but the VBN of GBPs was already over 20 mg% at the 15°C storage temperature. A VBN value of 20 mg% is the prescribed point of spoilage according to the Korean Food Standards Codex. (Korea Food & Drug Administration, 2012). Likewise 5°C storage temperature did not much affect the decomposition of GBPs, but VBN was increased up to 19.3 mg% during the storage period (Fig.

Table 1. Storage period of TTI and ground beef patty depend on storage temperature

Storage temperature (°C)	Storage time (h)		
	Initial stage	Middle stage	Final stage
25	0	12	24
15	0	96	192
5	0	144	288

Fig. 2. b^* -value of TTI or meat as storage temperature (A: 25°C, B: 15°C, C: 5°C). Vertical bars indicate standard deviations (n=3).

Fig. 3. Change in color of TTI and ground beef patty during storage periods.

4C). In this study, storage temperature was determined to be a more influential factor in increasing VBN than storage time.

Fig. 4. VBN value of GBPs as storage temperature (A: 25°C, B: 15°C, C: 5°C). Vertical bars indicate standard deviations (n=3).

pH

In this study, the pH of GBPs stored at 25°C ranged between 5 and 5.4 during storage periods and there was no big difference, but there was significant difference during storage period ($p < 0.05$) (Fig. 5). At 15°C storage temperature, initially the pH of GBPs was decreased and then increased up to 6.9 from middle stage to final stage. However, the pH of GBPs was not changed at 5°C during storage periods ($p > 0.05$). And the pH of GBPs stored at 15°C storage temperature as storage periods up to 6.77. Fresh meat is generally regarded as having a pH of 5.5-5.8 during storage. When pH of meat reaches pH 8, meat is regarded as being fully decomposed (Shin *et al.*, 2006). GBPs kept at 15°C were almost spoiled at final stage of storage period based on their pH. The GBPs kept at 5°C were not decomposed because of the low storage temperature. GBPs kept at 25°C were also not changed significantly because the storage periods was too short for sufficient chemical reaction of free amino acids and proteolysis by microorganism to take place, although the temperature was high

Fig. 5. pH of GBPs kept at various temperatures during storage periods. Vertical bars indicate standard deviations (n=3). Means with different letters are significantly different ($p < 0.05$).

enough to affect other quality parameters of GBPs (Kim and Lee, 2011). Low storage temperature ($< 5^\circ\text{C}$) did not greatly affect pH as previously decomposed in several

studies (Jeong *et al.*, 2006; Jeremiah and Gibson 2001). Holley *et al.* (1994) also observed that the pH of fresh meat increased with the storage period. Storage time is more influential in increasing the pH of food compared to storage temperature.

In the study of Shin *et al.* (2006), the pH of beef loin or pork belly kept 2°C was coincide with the freshness indicator which was pH sensitive. Likewise, the a^* -value of TTI kept at 15°C was associated with pH in this study. However, other TTI treatments at 25°C and 5°C were not matched with pH of GBPs. The a^* -value of TTIs kept 25°C rose quickly up to 26 with a red color, which indicated the spoilage stage, but the pH remained within the stable region, under 5.5. In the case of the 5°C TTI treatment, there was no change in either the a^* -value (Green TTI color) or the pH until the final stage, the point at which the VBN of GBPs was determined to be 20 mg%.

Water holding capacity

Water holding capacity ranged from 65% to 75% (Fig. 6). Even if there was no significant difference among samples or storage periods ($p>0.05$), samples stored at higher temperature had relatively higher water holding capacity during storage periods. Generally, water holding capacity is affected by storage temperature, rigor development, pH decline rate, ionic strength, and oxidation. In other words, isoelectric point (pI), low pH, or high storage temperature induce a reduction in water holding capacity (Huff-Lonerger and Lonergan, 2005). In this study, although the pH of GBPs was maintained at 25°C was in pI zone, water holding capacity of GBPs was not changed significantly at all storage temperature furthermore it was even higher than others during storage periods ($p>0.05$). At the final

Fig. 6. Water-holding capacity GBPs kept at storage temperatures during storage periods. Vertical bars indicate standard deviations ($n=3$). Means with different letters are significantly different ($p<0.05$).

storage period, although the pH of GBPs was stable during storage period at 25°C, water holding capacity was decreased to 66%, which was one of the lowest value. The reason was that the storage period (24 h) at 25°C was likely too short to affect the pH of the GBPs properties.

Microbial observation

Total microbial count of GBPs was counted during storage periods at 5°C, 15°C, and 25°C (Fig. 7). Initial spoilage is important when we observe the behavior of microorganism. Although initial total microbial count was quite high to do microbial test in all GBPs, the difference was a clearly observed among GBPs in this study. As depicted in Fig. 7, microorganisms significantly and gradually increased at 15°C and 25°C storage temperature during storage periods ($p<0.05$). Whereas the population of the 5°C treatment was relatively well controlled until 96 h storage however, it was dramatically increased after reaching 7

Fig. 7. Total microbial counts of GBPs during storage periods. Vertical bars indicate standard deviations ($n=3$). Means with different letters are significantly different ($p<0.05$).

Log CFU/g ($p < 0.05$). In the study of Jeong *et al.* (2006) two grades of beef were stored at 1°C. Total bacterial count reached 8 Log CFU/g at 28 d of storage. In the study of Shin *et al.* (2006), beef loin and pork belly were kept at 2°C for 10 d. Total aerobic bacteria of beef loin was 8 Log CFU/g at 10 d. Jeong *et al.* (2006) and Shin *et al.* (2006) supposed that the increase of microorganisms was temperature dependant.

However, each GBP sample was determined as 7 to 8 Log CFU/g at the middle stage of VBN development. Generally, over 8 Log CFU/g of microorganism make food to be decomposed and cause putrefied flavor which is unfavorable (KFDA, 2012). Therefore, the total microbial count cannot be observed through the basic senses until the meat is in the final stages of spoilage.

TTI color, VBN or Total microbial count of meat, as described by Arrhenius equation

Constant reaction rate (k) and activation energy (E_a) of TTI (color, a^* -value), quality of GBPs (VBN) or microbial growth (total microbial count) depend on storage temperature, and this was calculated through the Arrhenius equation (Fig. 8 and Table 2). The k of TTI color, VBN,

Fig. 8. Arrhenius plot of the constant reaction rate (k) of TTIs with various storage temperatures.

Table 2. Arrhenius parameters, activation energy (E_a) and Constant reaction rate (k) of TTI, VBN or total microbial count with different storage temperature

	T (°C)	k	E_a (kJ/mol)	R^2
TTI	25	0.099		
	15	0.008	95.1	0.819
	5	0.006		
VBN	25	0.685		
	15	0.396	92.6	0.910
	5	0.048		
Total microbial count	25	0.090		
	15	0.011	93.9	0.909
	5	0.006		

and total microbial count each developed at increasing rates with increasing storage temperature. The k of VBN was highest at every storage temperature. As a result of VBN determinations, every GBPs started to be spoiled at near middle stage of each samples in this study. The E_a of TTI, VBN, and total microbial count were similar as 95.12, 92.60 or 93.90 kJ/mol, respectively. The E_a of VBN and microbial growth were in the range of typical E_a values for food quality losses (Labuza, 1982). If there is more than ± 25 kJ/mol of E_a differences between food and TTI, the accuracy of indicator is regarded as poor (Park *et al.*, 2013; Taoukis, 2001; Wanihsuksombat *et al.*, 2010). Moreover, the E_a of food is changed depending on the temperature interval (Wanihsuksombat *et al.*, 2010). In this study, even though E_a was similar between the TTI and the determined quality parameters of GBPs, it did not coincide with the decomposition or spoilage of GBPs. This problem has been a major disadvantage of enzymatic TTIs for food shelf life in the past, but such systems may best fitted to their optimum foods as well as reverse engineered to some degree. (Byeon *et al.*, 2009; Kim *et al.*, 2012a; Kim *et al.*, 2012b; Park *et al.*, 2013; Taoukis *et al.*, 1999).

Conclusions

It was investigated whether a TTI was appropriate for the prediction of quality of GBPs, such as VBN or total microbial count at 25°C. However, there was insufficient correlation with the color change of the TTI at 5°C and at 15°C, although the interrelation between the TTI and the index of GBPs quality such as pH, VBN, and total microbial count was slightly observed. In other words, one type of TTI could not indicate quality of GBPs kept in various storage conditions. It has been suggested that TTIs should be designed individually depending on storage temperature, time, and the type of meat. Moreover, meat quality must be investigated because it may not be possible to simultaneously match various attributes of meat quality with the reaction of a single TTI.

Acknowledgements

This work was performed as a research project of the iPET (Korea Institute of Planning and Evaluation for Technology of Food, Agriculture, Forestry and Fisheries) supported by Ministry for Food, Agriculture, Forestry and Fisheries of Korea. This study was also partially supported by the SMART Research Professor Program of Konkuk University, Seoul, Korea.

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(Redeived 2013.4.17/Revised 2013.6.21/Accepted 2013.7.5)