Use of smart photochromic indicator for dynamic monitoring of the shelf life of chilled chicken based products

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**A R T I C L E   I N F O**

Article history:
Received 18 June 2013
Received in revised form 14 September 2013
Accepted 4 November 2013

Keywords:
Smart packaging
Temperature
Poultry products
Quality control

**A B S T R A C T**

This study evaluated the applicability of a photochromic time temperature indicator (TTI) to monitor the time-temperature history and shelf life of chilled boneless chicken breast. The results showed that the smart indicator showed good reproducibility during the discoloring process in all the conditions investigated. The response was not only visibly interpretable but also well adaptable to measurement using appropriate equipment. For an activation configuration of 4 s of ultraviolet light (UV) per label, the TTI’s rate of discoloration was similar to the quality loss of the meat samples analyzed. Thus, the photochromic label (4 s UV/label) attached to the samples set out to be a dynamic shelf-life label, assuring consumers the final point of quality of chilled boneless chicken breast in an easy and precise form, providing a reliable tool to monitor the supply chain of this product.

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1. Introduction

Temperature is considered the main factor affecting the quality and safety of perishable food products such as meat and chicken (Jedermann, Ruiz-Garcia, & Lang, 2009; Kreyenschmidt, Christiansen, Hubner, & Petersen, 2010; Raab et al., 2008; Smolander, Alakomi, Ritvanen, Vainionpaa, & Ahvenainen, 2004).

To slow the growth of microorganisms and extend the shelf life, the cold chain is widely used in the poultry products market (James, 1996; James, Vincent, Andrade Lima, & James, 2006; Likar & Jevsnik, 2006). However, temperature control during transport, distribution and storage (commercial and domestic) is often flawed, with different conditions from those recommended by the manufacturer (0 to 4 °C), with values that can pass 15 °C (Cárdenas, Giannuzzi, & Zaritzky, 2008; Limbo, Tori, Sinelli, Franzetti, & Casiraghi, 2010; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008; Rokka, Eerola, Smolander, Alakomi, & Ahvenainen, 2004; Vainionpää et al., 2004; Zhang et al., 2012).

The difficulty of ascertaining the real history of food temperature makes it difficult to predict their shelf life (Shimoni, Anderson, & Labuza, 2001). Thus, an emerging technology used to ensure the validity of food products is the use of smart packaging containing time-temperature indicators (Mai et al., 2011), systems that reflect, in a visual manner, the storage temperature conditions during the shelf-life of the food products to which they are attached (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2005; Kerry, O’Grady, & Hogan, 2006; Taoukis & Labuza, 1989).

The OnVu™ TTI B1 is an indicator of irreversible time and temperature used for food under refrigeration, whose operation is based on a photochromic reaction in the solid state. Its smart ink changes color from colorless to blue upon irradiation with ultraviolet light (activation). Once activated the ink reverts to the colorless state at a rate that is dependent on time and temperature (Kreyenschmidt et al., 2010; Mai et al., 2011; Verdade, 2010).

The end of the shelf life of the OnVu™ TTI B1 is defined as the time it takes the blue color of the label to reach a reference color (Mai, 2010; Verdade, 2010). The discoloration is proportional to the amount of light used in the charging process and can be adjusted by controlling the pulse duration and intensity of UV light used to activate the photochromic pigment. The bleaching process of the TTI has to be calibrated to take into account the characteristics, especially the expiry date of the food where the indicator will be placed (De Jong et al., 2005; Kreyenschmidt et al., 2010).

Given the above information, one technological possibility to control the temperature during the supply chain of chicken would be the use of smart packaging. Therefore, the objective of this study was to analyze the applicability of OnVu™ TTI B1 to monitor in real time the time-temperature history and shelf life of chilled boneless chicken breast.

2. Materials and methods

2.1. Characterization of the time-temperature indicator

The time and temperature sensor examined was OnVu™ label B1 (BASF patent WO/2006/048412). A manual ultraviolet (UV) charger developed for OnVu™ (GLP TTI, Bizerba, Germany) was used to activate the labels. The charger is equipped with high-power LEDs and a timer for activation times between 0.1 (minimum) and 60 s (maximum).
Once charged, the labels were covered with an optical filter in the form of a thermal transfer tape to protect them from re-charging by sunlight. The exposure time of the UV irradiation in the label was expressed in seconds (s).

2.1.1. Estimation of the shelf life of the TTI

Six different charging times were investigated to examine the influence of activation time (exposure to ultraviolet light) on the process of discoloration of the label.

The labels were activated under the following conditions: 1, 2, 3, 4, 5 and 6 s of UV light. After activation, the labels were attached on pre-cooled glass plates and stored in an incubator of high precision temperature (Model MA 415/S, Marconi, São Paulo, Brazil) at 3.0 ± 0.5 °C, whose internal temperature was monitored every 2 min by data collectors (Data Logger DHT5012, Perceptr, São Paulo, Brazil).

The room temperature during the activation procedure was controlled at 20.0 ± 1.0 °C. The analyses were performed twice and each activation time was evaluated in triplicate.

The discoloration of the labels was measured daily by using a colorimeter (Chroma Meter Model CR-400/410, Konica Minolta, Osaka, Japan) by the CIELab system (illuminant D65) obtaining the size of the $b^*$ chroma, a coordinate that quantifies the change of color from yellow ($b^* +$) to blue ($b^* -$). The analyses were discontinued when $b^* = 0$ (Fig. 1A), where no additional change in blue color could be measured in the label.

The end of the shelf life of the TTI was defined as the time it takes the dark blue color of the immediately charged label (Fig. 1C) to reach a light blue, almost gray color ($b^* = -7.0$), considered in this study as the last stage of the visually detectable blue color (Fig. 1B).

2.1.2. Reproducibility of the activation process of TTIs

The reproducibility of the activation process was measured in one hundred and twenty six (126) labels, twenty one (21) for each activation time, corresponding to seven evaluations of charge condition in triplicate. The labels were charged and immediately evaluated using a colorimeter, by the CIELab color system, obtaining the size of $b^*$ chroma.

2.2. Monitoring the shelf life of poultry products contained in smart packaging

Microbiological analyses of muscle and discoloration measurements of time and temperature indicators attached to the samples packaging were performed. At the end of the expiry date of the meat products, a comparison between the response rate of the labels (under specific activation condition) and the loss of food quality was performed.

![Fig. 1.](image)

(A) photochromic brand color when $b^* = 0$; (B) TTI color defined as the end of indicator shelf life, $b^* = -7.0$; (C) Dark blue tone presented by the label immediately after activation with UV light.

2.2.1. Preparation of meat samples

The raw material used was chilled boneless chicken breast, provided by two slaughterhouses under Federal Inspection Service (SIF), located in the state of Rio Grande do Sul/Brazil.

In the slaughterhouses, the samples followed the normal obtaining process; according to the production flow of the respective plants and after packing in the primary packages, they were attached to OnVu™ TTI B1 labels. The TTIs were activated following the best charge conditions evaluated in Section 2.1 for a maximum storage time of twelve days (shelf life of chilled commercial boneless chicken breast).

The products contained in the activated smart packaging were placed in refrigerated trucks for transport to their final destination, the retail market in the city of Rio Grande/RS/Brazil.

2.2.2. Inland logistics chain of fresh produce

The mapping of the temperature during the distribution of fresh foods was assessed in two routes of land transportation. The first from the city of Morro Redondo/RS/Brazil to Rio Grande/RS/Brazil (Route 1), totaling about 100 km, and the other, from Westfalia/RS/Brazil to Rio Grande/RS/Brazil (Route 2), about 430 km. The temperature was measured using the smart packaged meat products as reference (as per Section 2.2.1) and data collectors, which were applied to measure muscle and environment temperature in the refrigerated truck, respectively.

2.2.3. Storage simulation

After completing the journey, the products were unloaded at their final destination, and the samples were immediately transported in ice coolers, in less than 20 min, until the Laboratory of Food Technology of the Federal University of Rio Grande (FURG)/RS/Brazil.

In the laboratory, simulations of temperature conditions during storage in commercial premises (point of sale) and household refrigerator were performed. For this, the samples were stored in three high precision incubators for twelve days.

The temperatures evaluated were: 3.0 (ideal situation), 7.0 and 10.0 (abusive situation) ± 0.5 °C, and monitored every 2 min by data collectors. Products from the logistics Route 1 were stored at 3.0 and 10.0 ± 0.5 °C, while samples from Route 2 were maintained at 3.0 and 7.0 ± 0.5 °C.

The temperature of 3 °C was chosen because it is the recommended storage condition of chilled products (0 to 4 °C) (Brasil, 1998), while the others were based on work carried out by Zhang et al. (2012), Limbo et al. (2010), Cárdenas et al. (2008), Vainionpää et al. (2004) and Rokka et al. (2004), whose studies featured temperatures around 7 and 10 °C as the temperature profiles that represent the actual conditions of the distribution chain of fresh meat products, from the producer to the final consumer.

2.2.3.1. Microbiological stability of boneless chicken breast

To characterize the loss of quality of the meat samples, microbiological analyses were conducted on boneless chicken breast at 0, 1, 4, 7, 10 and 12 days of storage for each stock condition, with the exception of Salmonella spp., performed only at time zero. All analyses were performed in triplicate.

The choice of microorganisms for this study was based on literature (Barbut, 2002; Chouliara, Badeka, Savvaidis, & Kontominas, 2008; Davies & Board, 1998; Franco & Landgraf, 2008; Jay, 2005; Patsias, Badeka, Savvaidis, & Kontominas, 2008) and Brazilian legislation (Brasil, 1998). Analyses were performed using the total count of psychrotrophic aerobes; enumeration of Staphylococcus spp.; determination of thermotolerant coliforms at 45 °C and Salmonella spp.

A sample (25 g) was taken aseptically from the boneless chicken breast, transferred aseptically to a stomacher bag (Seward Medical, London, UK) containing 225 mL of sterile 0.1% peptone water and homogenized using a stomacher (Lab Blender 400, Seward Medical) during 60 s at room temperature, then serial dilutions were prepared in sterile 0.1% peptone water for the continuation of the analysis. For the enumeration of psychrotrophic microorganisms, Staphylococcus
spp. and the detection of *Salmonella* spp. the classical methodology according to American Public Health Association (APHA, 2001) was used. Thermotolerant coliforms at 45 °C were investigated according to the official methodology of the Ministry of Agriculture, Livestock and Supply (MAPA) (Brasil, 2003).

2.2.3.2. Discoloration of TTIs. The process of discoloration of the labels was measured by using a colorimeter, by the CIELab color system (illuminant D65), monitoring daily, in triplicate, the value of *b* chroma for twelve days of storage.

2.2.4. Data analysis

Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) was used to calculate mean values, standard deviations and construct graphs.

The results of the *b* chroma values measured in smart labels underwent Quantitative Descriptive Analysis, through the program Statistica 7.0, using analysis of variance (ANOVA) and subsequent Tukey’s test, with significance level of 5% (*p* < 0.05).

### 3. Results and discussion

3.1. Characterization of the time–temperature indicator

Table 1 shows the mean values for the color parameter *b* during the evaluation of the reproducibility of the activation process of the labels. In Table 1 it can be seen that the intensity of the blue color for the labels activated during 1 s at 20 °C was significantly lower than all other charging conditions, with values of *b* chroma significantly (*p* < 0.05) higher than those measured in the other charging situations. This phenomenon is explained by Bamfield (2001), whose studies showed that low exposure times of photochromic compounds to UV light cause a loss of optical density of its colored state, making its hue less intense.

Fig. 2 shows the mean deviations (σ) of the *b* values measured during the evaluation of the reproducibility of the activation process of smart labels.

<table>
<thead>
<tr>
<th>Activation time (s)</th>
<th>Chroma b*</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-25.48 ± 0.39a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-28.97 ± 0.30b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-29.82 ± 0.36b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-29.29 ± 0.28b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-29.64 ± 0.55b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-29.14 ± 0.31b</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Reproducibility of the charging process of TTIs, assessed by the mean deviation of *b* values.

3.2. Monitoring the shelf life of poultry products contained in smart packaging

3.2.1. Inland chain logistics of fresh produce

Fig. 4 shows temperature results measured by the data loggers for monitoring the logistic routes.

During the Route 1 no temperature deviation was observed, and the values measured in this way showed results below the Brazilian standards, 4.0 ± 1.0 °C (Brasil, 1998). A different situation was observed in Route 2, in which the temperature of the truck stood still at approximately 8 °C for 2 h. These deviations have coincided with the start of delivery in the retail market of the city of Rio Grande/RS/Brazil, caused by opening the trunk of the truck. However, the oscillations did not affect the temperature of the products, which are presented as below 4 °C during the entire trip.

Similar results were found by Rokka et al. (2004) and Smolander et al. (2004), who described temperature variations in the logistics chain of chilled cuts of chicken in the Republic of Finland from 2.9 to 8.3 °C.
Table 2 shows the color results measured instrumentally and visually on the TTIs attached to samples before and after transport. Observe that in each activation condition the color of the labels showed no significant difference (p < 0.05), results that corroborate with the temperature values measured in the products after distribution, as temperatures over 4 °C were not observed at the meat samples.

### 3.2.2. Storage simulation

#### 3.2.2.1. Microbiological stability of boneless chicken breast

All samples were negative for *Salmonella* spp., indicating good quality hygiene programs of farms and hatcheries, as well as appropriate hygienic and sanitary conditions during slaughter and processing of carcasses in the slaughterhouses in study. Despite the absence of *Salmonella* spp. in the samples, several researchers have found a high incidence of this bacterium in chilled and frozen chicken carcasses, with results ranging from 12 to 59% of positivity (Borsoi, Moraes, Salle, & Nascimento, 2010; Carvalho & Cortez, 2005; Chen et al., 2010; Fuzihara, Fernandes, & Franco, 2000; Reiter, Fiorese, Moretto, López, & Jordano, 2007; Ribeiro, Kellermann, Dos Santos, Bessa, & Nascimento, 2007). The variation of results among other quantifying studies makes ongoing monitoring of the pathogen relevant.

### Table 2

<table>
<thead>
<tr>
<th>Values of b*</th>
<th>4 s/label&lt;sup&gt;1&lt;/sup&gt;</th>
<th>5 s/label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route 1 Before&lt;sup&gt;2&lt;/sup&gt;</td>
<td>−29.39 &lt;small&gt;a ± 0.38&lt;/small&gt;</td>
<td>−29.97 &lt;small&gt;c ± 0.65&lt;/small&gt;</td>
</tr>
<tr>
<td>After&lt;sup&gt;2&lt;/sup&gt;</td>
<td>−29.12 &lt;small&gt;a ± 0.22&lt;/small&gt;</td>
<td>−29.71 &lt;small&gt;c ± 0.34&lt;/small&gt;</td>
</tr>
<tr>
<td>Route 2 Before&lt;sup&gt;2&lt;/sup&gt;</td>
<td>−29.56 &lt;small&gt;b ± 0.31&lt;/small&gt;</td>
<td>−29.68 &lt;small&gt;d ± 0.40&lt;/small&gt;</td>
</tr>
<tr>
<td>After&lt;sup&gt;2&lt;/sup&gt;</td>
<td>−29.18 &lt;small&gt;b ± 0.72&lt;/small&gt;</td>
<td>−28.87 &lt;small&gt;d ± 0.09&lt;/small&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>b* values measured at the time of charging in a manufacturing plant.

<sup>2</sup>b* values observed after transport logistics.

<sup>3</sup>Activation conditions of TTIs.

Mean values marked with the same letter within the same column were not significantly different (p < 0.05) by Tukey test.
Fig. 5 shows the results of microbiological enumeration of psychrotrophic, aerobic micro-organisms (Fig. 5X), *Staphylococcus* spp. (Fig. 5Y) and thermotolerant coliforms (Fig. 5Z) in samples stored under different temperature conditions for twelve days of storage.

Although the count of aerobic psychrotrophic microorganisms indicate the degree of deterioration of refrigerated poultry meat (Patsias et al., 2008), Brazilian law establishes no standards for these microorganisms. However, the International Commission on Microbiological Specifications for Foods — ICMSF (1978) considers counts in excess of $10^6$ (6 log10) to $10^7$ (7 log10) of colony forming units (CFU)/g for this group of bacteria out of range of the ideal sanitary conditions of meat products. Thus, given the standard of 7 log10 CFU/g, samples kept in optimum conditions of storage (3 °C) reached counts below those aforementioned by the tenth day of shelf life, while the product under isothermal conditions at 10 °C and 7 °C showed higher values than the standards before the fourth and fifth days of storage, respectively (Fig. 5X).

The results for the analyses of thermotolerant coliforms (Fig. 5Z) at time zero were less than 3 CFU/g; low counts of these microorganisms indicate good sanitary conditions of food and eliminate the suspicion of the presence of pathogens of enteric origin, coming from the same source of contamination (Dickens, Ingram, Hinton, & A., 2004; Franco & Landgraf, 2008). Following the Brazilian microbiological standard for thermotolerant coliforms at 45 °C (Brasil, 2001), the only one for chicken, of $\leq 10^4$ (4 log10) CFU/g, the samples stored at 3, 7 and 10 °C remained suitable for consumption until the tenth, fifth and third day of storage, respectively.

*Staphylococci* are microorganisms forming part of the raw meat microflora of poultry (Russell, 2008). Work performed by Franco and Landgraf (2008) and Barbut (2002) reported that between $10^5$ (5 log10) and $10^6$ (6 log10) colony forming units of *Staphylococcus* per gram of food are required so that the toxin is formed at levels capable of causing intoxication. Considering this range, only the samples stored at 10 °C from the fourth day of storage could pose a risk to consumer health, if the strain were enterotoxigenic (Fig. 5Y).

Studies by Zhang et al. (2012), Cortez-Vega, Pizato, and Prentice (2012) and Porto, Tôrres, Ilha, Luiz, and Santanna (2000) found higher counts of these microorganisms in samples of refrigerated chicken.
breast, with aerobic psychrotrophs and enumeration of *Staphylococcus* spp. 10\(^{6}\) CFU/g in five days of storage at 4 °C and about 10\(^{10}\) CFU/g when kept at 10 °C for four days. The incidence of bacteria can be explained by the high initial microbiota in the products analyzed (time zero), since the shelf life of the meat has an inverse relationship with its initial contamination (Barbut, 2001; Jay, 2005; Jay, Loessner, & Golden, 2005).

The low prevalence of the analyzed bacteria at time zero indicated good process control through the steps of obtaining products, with effective application of the Good Manufacturing Practices (GMPs) by the slaughterhouses evaluated. However, the samples did not remain fit for consumption until the end of the twelfth day of validity (as supplied by the manufacturer in the package). The microbiological shelf life of products at 3 °C was ten days. It is up to the manufacturers to reassess the validity of this commercial product.

The microbiological analysis showed that the growth of the bacterial microflora of the meat was significantly (*p* < 0.05) delayed when maintained at lower temperatures. The boneless chicken breast obtained a shelf life of ten days under ideal conditions of storage (3 °C), while samples stored at 7 °C should be consumed before reaching five days of validity and those kept at 10 °C from the third day of storage already represent a health risk if consumed.

### 3.2.2.2. Discoloration of TTIs

**Fig. 6** shows the color results that characterize the discoloring process of labels attached to the packaging of meat samples, activated for 4 s (**Fig. 6X**) and 5 s (**Fig. 6Y**) of UV light, respectively.

The color change of the label was significantly accelerated during storage at temperatures exceeding ideal behavior, which according to the GMPs applied by the slaughterhouses evaluated.

**Fig. 6.** Discoloration presented by the labels — activation condition: (X) 4 s; (Y) 5 s — during storage: (A) 3 to 10 °C and (B) 3 and 7 °C. Where *b* = −7.0 represents the color defined as the end of TTI shelf life. The error bars represent the standard deviation of triplicates for each measurement.

**Fig. 7.** Color observed in the TTIs (activation condition: 4 s at 20 °C) attached to the product during the twelve days of validity, kept under optimal (3 °C) and abusive (7 and 10 °C) temperatures.

**Coelho (2006)** and **Crano and Guglielmetti (2002)**, characterizes the operating principle of the active compounds present in TTIs.

The color response of labels obtained good reproducibility during all storage conditions, with low deviations between the values of triplicate measurements for each charging condition.
Table 3

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Shelf life of chicken cuts (days)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiologically*</td>
<td>5.4°C, 6.6°C, 7.7°C</td>
<td>Smolander et al. (2004)</td>
</tr>
<tr>
<td>Fresh-check C628</td>
<td>12 12 9</td>
<td></td>
</tr>
<tr>
<td>Fresh-check HC63</td>
<td>&gt;12 &gt;12 12</td>
<td></td>
</tr>
<tr>
<td>Fresh-check JC221-1</td>
<td>12 9 7</td>
<td></td>
</tr>
<tr>
<td>VITASB M2-15</td>
<td>5 5 5</td>
<td></td>
</tr>
<tr>
<td>VITASB M2-21a</td>
<td>&gt;12 &gt;12 12</td>
<td></td>
</tr>
<tr>
<td>VITASB M2-21b</td>
<td>&gt;12 &gt;12 12</td>
<td></td>
</tr>
<tr>
<td>VITASB C2-10</td>
<td>7 7 7</td>
<td></td>
</tr>
<tr>
<td>VITASB C2-13</td>
<td>&gt;12 &gt;12 12</td>
<td></td>
</tr>
<tr>
<td>3M</td>
<td>3.0°C 7.0°C 10.0°C</td>
<td>This work</td>
</tr>
<tr>
<td>Microbiologically*</td>
<td>10 ≤&lt;5 3 3</td>
<td></td>
</tr>
<tr>
<td>OnVu™ (activation 4 s)</td>
<td>10–11 4–5 3</td>
<td></td>
</tr>
<tr>
<td>OnVu™ (activation 5 s)</td>
<td>12 5–6 3–4</td>
<td></td>
</tr>
</tbody>
</table>

* Shelf life estimated considering enumeration of aerobic mesophile bacteria of ≤10⁷ CFU/g
b Shelf life estimated considering enumeration of aerobic psychrotrophic bacteria of ≤10⁷ CFU/g and thermotolerant coliforms at 45°C ≤10⁷ CFU/g

In Fig. 7, the different shades of blue observed in the smart indicators of the photochromatic brand OnVu™ TTI B1 activated during 4 s are shown.

The color response of TTIls was both visually understandable (Fig. 7) and adaptable in measuring equipment (Fig. 6) reliability and reproducibility. Results corroborate with those found by Kreyenschmidt et al. (2010) and Verdade (2010) for other charge conditions of the same intelligent indicator.

The TTIls activated by 4 s at 20 °C showed a discoloration similar to the rate of deterioration of the meat product analysis, with expiry very close in all temperature conditions evaluated. Other researchers have also pointed to results with good correlation between the discoloration of TTI OnVu™ B1 (activation conditions from 0.65 to 3.0 s of UV light) and deterioration of marine products (Ma et al., 2011; Tsiironi et al., 2011).

Evaluating studies conducted with poultry products (Smolander et al., 2004; Vainionpää et al., 2004) using other commercially available smart indicators of time and temperature (Fresh-Check, VITASB and 3M TTI), it is observed that the correlations between the shelf life of the meat samples and the expiration of the intelligent indicators in this study were best, being almost coincident (Table 3). Thus, the implementation of TTI OnVu™ B1 combined with production management systems can provide an effective tool to ensure reliable delivery of safe food.

4. Conclusion

The color response of photochromatic indicators attached to meat products was visually interpretable and adaptable to measurement using appropriate equipment, with good reproducibility during the discoloring process in all temperature conditions studied.

Considering the ten days shelf life of the samples, the best condition for activation of TTIls was 4 s of UV light at 20 °C. With this charge, the intelligent indicator showed a discoloration similar to the rate of deterioration of the analyzed product, setting a dynamic shelf-life label, offering consumers the final point of quality of chilled boneless chicken breast easily and accurately, constituting a reliable tool to monitor the supply chain of this product.

Acknowledgments

The authors thank the BASF Company for the technical support and supply of smart labels, the slaughterhouses that opened their doors and provided the meat raw materials and the Ministry of Agriculture, Livestock and Supply (MAPA) for their support throughout the development of this work.

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