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Dynamic monitoring of the shelf life of Cobia (*Rachycentron canadum*): a study on the applicability of a smart photochromic indicator

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Summary To ensure the marketing of fresh fish-based products, it is necessary to develop fast methods that assess its freshness in real time. This study therefore evaluated the applicability of a photochromic time–temperature indicator (TTI) to monitor the time and temperature history during the period of validity of the whole fish of the cobia specimen stored in ice. The TTI response was both visibly interpreted as well as adaptable to measurement using suitable equipment. The results showed that the smart indicator activated during 6 s of ultraviolet light showed a similar rate of deterioration of the analysed product visual response, proving to be a dynamic shelf life indicator that can assure consumers the ultimate quality point of the entire cobia easily, cheaply and accurately.

Keywords Freshness, quality control, temperature, whole fish.

Introduction

Cobia (*Rachycentron canadum*) belonging to the family Rachycentridae is a widely distributed pelagic fish, particularly in tropical, subtropical and warm temperate waters. In Brazil, cobia is a fish species of high cultivation and marketing potential (Benetti *et al*., 2010). The reason for their success in farming is their fast growth rate, weighing about 5–6 kg within 1 year and 8–10 kg in 16 months. Another reason is that it is considered a very good fish for eating. The meat of cobia has very distinctive qualities, and it is used mostly as sashimi or in more sophisticated dishes (Robinson *et al*., 2012).

For being consumed preferably raw, the effective temperature control during these products distribution is of utmost importance to ensure the quality of the product offered and essential for its commercial viability. Therefore, it is necessary to apply new quality systems that easily and reliably perform continuous monitoring of storage conditions, from production to consumption (Tsironi *et al*., 2008; Brizio, 2014).

In this sense, the use of smart time–temperature indicators (TTI) has been an emerging technology with great potential to continuously monitor the shelf life of foods (Galagan & Su, 2008; Galagan *et al*., 2010; Mai *et al*., 2011a; Brizio & Prentice, 2014).

The OnVu® TTI B_1 is an irreversible time–temperature indicator whose operating principle is based on a photochromic solid-state reaction. Its smart ink changes colour from colourless to blue after irradiation with ultraviolet light (UV) (activation). Once activated, the ink reverts to the colourless state to a response rate that is dependent on time and temperature of storage on the label (Kreyenschmidt *et al*., 2010; Mai *et al*., 2011b).

The response rate (discoloration) of *OnVu*® TTI B_1 is inversely proportional to the amount of UV light used during its activation (Brizio & Prentice, 2014) and must be calibrated taking into account the characteristics of the food where the indicator will be applied (Kreyenschmidt *et al*., 2010).

Thus, an innovative alternative in the dynamic control of fresh whole fish chain could be the use of a smart label directly attached to the animal's skin. Therefore, this study aimed to examine the applicability of *OnVu*® TTI B_1 in monitoring the quality and shelf life of the whole cobia fish specimen during simulation of the storage and distribution chain in ice.

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Materials and methods

Characterisation of the raw material

For this research, cobia specimens obtained from a near-shore production of about 700 m from the Recanto da Lagoinha beach in Ubatuba, São Paulo, Brazil, were used. The cobia specimens were harvested and slaughtered by thermal shock. Immediately after slaughter, the fish were transported to the analytical laboratory in boxes containing ice (0–4 °C). The analysis started in less than 24 h after harvesting.

Characterisation of photochromic indicator of time and temperature

The time–temperature indicator analysed was *OnVu*® label B_1 (BASF, Patent WO/2006/048412). This system is a nontoxic printable time–temperature indicator, whose reaction is based on a photochromic solid-state reaction (AMES test negative, LD50 > 2000 mg kg^{-1}). 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).

The exposure time of the UV irradiation label was expressed in seconds (s). In this study, five different load times (5, 6, 7, 8, 9 and 10 s of UV light) were investigated to examine the influence of activation time on the process of discoloration of TTI and thus set the best activation condition.

During the activation procedure of TTIs, room temperature was controlled at 20.0 ± 1.0 °C, because according to Kreyenschmidt *et al.* (2010), changes in environmental conditions influenced the activation process of this type of label.

Once loaded, the labels were covered with an optical filter in the form of a thermal transfer ribbon (material supplied by the labels manufacturer) to protect them from reloading by sunlight and immediately glued to the skin of the cobia fish specimen.

Reproducibility of the activation process of TTIs

The reproducibility of the activation process was measured in a total of one hundred and twenty-six (126) stickers, twenty-one (21) TTIs for each activation time, corresponding to seven evaluations by load condition in triplicate. The labels were loaded and immediately evaluated by using a colorimeter (Chroma Meter Model CR-400/410, Konica Minolta, Osaka, Japan) by the CIELab system (illuminant D_{65}), obtaining the scale of chroma b*, a coordinate that quantifies the change of colour from yellow (b* +) to blue (b* −).

The results of the chroma b* values measured in each activation condition underwent quantitative descriptive analysis, through the Statistica 7.0 program using analysis of variance (ANOVA) and subsequent Tukey's test, with significance level of 5% ($P < 0.05$).

Estimation of the shelf life of cobia during simulation of storage on ice

The TTI-fish sets were placed in cool boxes containing ice (ice:fish ratio of 2:1 w/w). Daily, addition of ice was held in the boxes to maintain the temperature in the range of 0.5–2 °C, which was monitored continuously by data collectors (Data Logger DHT5012, Perceptec, São Paulo, Brazil).

During twenty-two days of storage, traditional analysis of characterisation of fish quality and measurements of discoloration of time and temperature indicators (activation conditions 5, 6, 7, 8, 9 and 10 s UV light) attached to samples were made. At the end of the validity of the fish, a comparison between the response rate of TTIs and the loss of food quality was performed, to find the appropriate TTI loading condition for use in whole cobia fish.

Conventional analysis of characterisation of quality

Sensory stability of the whole fish

Ten previously trained judges participated in the testing panel. Sensory analyses were conducted in the fish after 1, 3, 5, 8, 10, 12, 15, 17, 19 and 22 days of storage on ice following the methodology proposed by Sant'Ana *et al.* (2011), with adaptations.

The quality index of the samples was obtained by evaluation of sensory characteristics related to skin (colour/appearance, presence of odour and mucus), eye (cornea, pupil and shape), gills (colour, shape, odour and mucus) and texture or elasticity of the abdominal musculature, assigning different scores ranging incrementally according to each attribute of unwanted changes during the storage time of the fish, as shown in Table 1.

Histological analyses of the fish muscle

The histological quality of the dorsal muscle of the fish was evaluated at times 1,7,14 and 21 days of storage on ice. The sample preparation was performed using the methodology suggested by Vitanov *et al.* (1995), and later, each sample was analysed by optical microscopy.

Microbiological stability of the fish muscle

Microbiological analyses of the muscle were performed on times 1, 4, 8, 12, 15, 19, 22 days of storage on ice.

The choice of microorganisms for this study was based on literature (Jay, 2005) and Brazilian legislation (Brasil, 2003). Analyses were performed with total count of psychrotrophic aerobes; Enterobacteriaceae; Pseudomonads and H_2S-producing bacteria.

A sample (25 g) was taken aseptically from the muscle of cobia, transferred aseptically to a stomacher bag (Seward Medical, London, UK) containing 225 mL of sterile 0.1% peptone water and homogenised 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, as per the official methodology of the Ministry of Agriculture, Livestock and Supply (MAPA) (Brasil, 2003) and American Public Health Association (APHA, 2001). All analyses were performed in triplicate, using three fish each day of analysis.

Table 1 Quality index method scheme developed of the cobia

Parameter		Description	Score
Skin	Colour/ appearance	Iridescent pigmentation (black-silver), shining	0
		Shining reduction (some reduction on black)	1
		Distinct reduction, faded colours	2
	Mucus	Transparent, not clotted	0
		Clotted and milky	1
		Clotted and yellow	2
	Odour	Fresh	0
		Neutral to metallic, dry grass, grains	1
		Acrid	2
		Rancid	3
Eyes	Cornea	Translucent	0
		Slightly opalescent	1
		Opaque, milky	2
	Pupils	Black, clearly outlined (rounded)	0
		Slightly opaque, outline reduction	1
		Grey, not outlined	2
	Form	Convex, protuberant	0
		Flat, plane	1
		Sunken, concave	2
Gills	Colour	Red/shining	0
		Pale red	1
		Grey-brown	2
	Mucus	Transparent	0
		Yellow, clotted	1
		Brown	2
	Odour	Fresh, seaweed	0
		Neutral, slightly seaweedy	1
		Slightly rancid	2
		Rotten	3
	Form	Complete, well defined	0
		Slightly deformed	1
		Deformed	2
Texture	Elasticity	Finger mark returns quickly (<2 s)	0
		Finger mark returns slowly (between 3 and 2 s)	1
		Finger mark returns slowly (>3 s)	2
Quality Index total			0–24

Source: Adapted from Sant'Ana *et al.* (2011).

Analysis of unconventional characterisation of quality – smart indicator

Discoloration of TTIs

The discoloration of the labels attached to the fish was measured daily using a colorimeter, through the CIELab system (D_{65} illuminant), whereby the dimension of the chroma b* was obtained. Analyses of the fish-TTIs set were discontinued after 22 days of storage in ice.

Following the work of Brizio & Prentice (2014), the end of the shelf life of the TTI was defined as the time it takes the dark blue colour of the label immediately loaded to reach a light blue, almost grey (chroma b* = −7.0), considered by the authors as the final stage of the visually detectable blue colour (Fig. S1).

The TTI kinetics

Following the methodology described by Taoukis & Labuza (1989), a kinetic evaluation of the behaviour of the TTI and the product was carried out. According to these authors, the smart indicator should have a response rate with a similar temperature dependence of the thermal dependence of the deterioration of the product that will be attached, that is the food and the indicator should present similar activation energies (Ea).

To calculate the activation energies (kcal mol^{-1}), Taoukis & Labuza (1989), based on the Arrhenius expression, propose that the colour response of TTIs and the response of traditional food quality analyses be expressed by Equation 1:

$$f(A) = K(T)t, \qquad (1)$$

where $f(A)$ represents a change in the quality index, K is the kinetic constant for the reaction rate (temperature dependent), and t expresses storage time.

To obtain the value of Ea, the kinetic expressions of TTI behaviour should be validated dynamically, that is in conditions of varying temperature (Taoukis & Labuza, 1989; Tsironi *et al.*, 2008). For this, the discoloration of ITT[1] was investigated under different storage temperatures (2–10 °C).

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 1.0, 7.0 e 10.0 ± 0,5 °C, whose internal temperature was monitored every 2 min by data collectors (Data Logger DHT5012, Perceptec, São Paulo, Brazil). The room temperature during the activation procedure was controlled at 20.0 ± 1.0 °C.

The process of discoloration of the labels was measured using a colorimeter, by the CIELab colour system (illuminant D_{65}), monitoring daily, in triplicate, the value of b* *chroma* for 18 days of storage.

Thus, correlating the colour response of TTIs as a function of time, the values of the constants K were obtained from the slope of the line. And later, taking the logarithm on both sides of the Arrhenius function, and drawing a relation between log K and $1/t$, the activation energy of the indicator was calculated.

Results and discussion

Conventional analysis of characterisation of quality

The Fig. S2 shows the result of sensory analyses performed on samples of cobia during simulation of storage on ice.

The results show that during the first 5 days of storage in ice, the attributes of the fish skin (Fig. S2a) suffered no unwanted changes. The mucus was the characteristic of minor relevance for sensory analysis. However, the attribute odour obtained increasing results throughout the storage period of the samples, and from day twenty of storage, it got high scores from the panel of judges (> 2), indicating loss of freshness.

The changes in the eyes of the animals were very remarkable during storage on ice (Fig. S2b). The judges pointed to the opalescent corneas and pupils and indistinct contours during the first days of storage; however, scores showing actual loss of quality were only recorded after the fifteenth day. Regarding the shape of the eye, this was convex on the first day of storage, becoming flat and smooth ten days thereafter, followed by a concave shape during the remaining storage period.

The attributes analysed in the gills of the cobia (Fig. S2) showed high scores during storage of fish. According to Sant'Ana *et al.* (2011) and Lauzon *et al.* (2010), the gill area is considered the part of the fish that is more susceptible to microbial decomposition, and therefore, the degree of deterioration can easily be seen when the characteristic bright red colour begins changing to brown and then to greyish-brown. A phenomenon that was noticed by the judges after ten days of storage, when the colour of the gills samples of cobia already had grey-brown colour accompanied by an odour that ranged from neutral to foul.

The elasticity of the muscles took longer to show visible change (Fig. S2d), and the mean scores for the abdomen quality attributes began the characterisation of loss of freshness from the seventeenth day of storage. Results that can be better characterised through Fig. S3 which shows the histological picture of the muscular fibres of the cobia samples.

According to Delbarre-Ladrat *et al.* (2006) muscle fibres of freshly slaughtered fish have well-defined hexagonal shapes with few extracellular spaces filled with collagen (Fig. S3a), which undergoes gradual degradation after the death of the animal. Thus, after 7 days of storage, an initial loss of muscle structure was observed, resulting in irregular and dehydrated muscular fibres (Fig. S3b). After fourteen days of storage in ice, the collagen fibres and connective tissue already had a disorganised structure (Fig. S3c), being visibly aggravated with storage time (Fig. S3d), where an increase of spacing between the muscle fibres and appearance of numerous cracks in its interior occurred. According to Chéret *et al.* (2006) and Ando *et al.* (1999), this degradation occurs because of the action of proteases and microorganisms and causes the weakening of the fish muscle, with consequent reduction of its stiffness.

The maximum demerit score in sensory analysis was set at 24 points, however, based on the results contained in Figs S2 and S3, and in studies conducted by Mach & Nordvedt (2012); Sant'Ana *et al.* (2011); Nuin *et al.* (2008); Nielsen & Green (2007) and Gonçalves *et al.* (2007), scores higher than 13 characterised a cobia fish in the state of deterioration, or unfit for human consumption. Thus, considering the above value, the samples should not be consumed from the sixteenth day of storage. However, as changes in sensory attributes are mainly a result of the activity of microorganisms (Olafsdóttir *et al.*, 1997), to reliably establish the shelf life of cobia, sensory results were analysed together with microbiological data on the growth of spoilage bacteria.

Figure S4 shows the microbiological results of enumeration of psychrotrophic aerobe microorganisms, *Pseudomonas* spp., Enterobacteriaceae and H_2S-producing bacteria on the cobia samples after twenty-two days of storage on ice.

During the first five days of storage, low bacterial counts were observed on the samples. These results corroborate with the findings in sensory analysis (Fig. S2) at the beginning of the period of storage of the fish.

From the eighth day of analysis, there was a remarkable acceleration in the growth of psychrotrophic aerobic bacteria and *Pseudomonas* spp. According to Jay *et al.* (2005), the main species responsible for deterioration of refrigerated products based on fish are included in the group of psychrotrophic aerobic bacteria, the reason why they have substantial importance in reducing their shelf life. Dalgaard *et al.* (1997) state that in fresh fish products, the deterioration is caused by the activity of a fraction of the total bacterial population, called specific spoilage organisms (SSO). In this study, the results proved that the bacteria *Pseudomonas* spp. were the main organisms responsible for the deterioration of cobia, thus are considered as SSO and were virtually the total aerobic bacteria present in the samples. The predominance of *Pseudomonas* spp. has also been reported for the deterioration of various species of fish by several researchers (Paleologos *et al.*, 2004; Nuin *et al.*, 2008; Sant'Ana *et al.*, 2011).

A group of Enterobacteriaceae and H_2S-producing bacteria were also observed in the samples, but only after 2 weeks of storage in significant amounts to cause early deterioration. According to Tejada *et al.* (2007), the contribution of these bacteria in the deterioration of fish of marine origin is lower compared to the genus *Pseudomonas* spp.

There is no consensus in the literature on bacterial counts associated with the time of sensory rejection in predicting the shelf life of fish-based products (Taoukis *et al.*, 1999; Koutsoumanis, 2001; Paleologos *et al.*, 2004; Ozogul *et al.*, 2006; Rodrıguez *et al.*, 2006; Sant'Ana *et al.*, 2011). However, the International Commission on Microbiological Specifications for Foods – ICMSF (1978) considers out of ideal sanitary conditions products that exceed bacterial counts of 10^6 (6 $\log_{10}$) to 10^7 (7 $\log_{10}$) colony-forming units (CFU) g^{-1}. Thus, considering the sensory results and given the maximum standard 6 $\log_{10}$ CFU g^{-1}, the samples would be suitable for consumption until the fifteenth day of validity (shelf life of 15 days) when stored on ice.

Unconventional analysis of characterisation of quality – smart indicator

Table 2 shows the mean values for the colour parameter chroma b*, while Figure S5 shows the deviations (σ) of the mean values of chroma b* measured during the evaluation of the reproducibility of the activation procedure of smart labels (activation conditions: 5, 6, 7, 8, 9 and 10 s of UV light).

In each condition analysed, the activation process showed no significant variation ($P > 0.05$), a result that ensures the reliability of the charging process. According to Shimoni *et al.* (2001), the reliability of a TTI system is a necessary and important characteristic for its implementation in the management of the cold chain. For the *OnVu*® TTI B_1 label, a process of reproducible load is required, as variations in activation lead to different times of duration (Kreyenschmidt *et al.*, 2010; Mai *et al.*, 2011b).

Figure S6 shows the results characterising the discoloration of the labels attached on the skin of the cobia fish specimen during storage in ice.

The labels showed a uniform discoloration during storage on ice, with low deviations (0.1–0.92) between the measured values of triplicates for each loading condition, and this corroborates with the findings by Brizio & Prentice (2014); Kreyenschmidt *et al.* (2010) for other load conditions of the same smart indicator.

Another feature to be highlighted was the proper adhesion of labels on the skin of the fish. The labels remained well attached to cobia samples for the twenty-two days of analysis. A feature that will facilitate the use of this TTI in fish marketed whole.

The discoloration of photochromic brand of TTIs showed a clear dependence and inversely proportional to the time of activation. The colour response was both visually understandable and adaptable to the measurement equipment. Thus, considering b* = −7.0 as a value that represents the last stage of the blue colour perceived visually, the labels had a duration of 12.5 (300 h), 15 (360 h), 18 (432 h), 21 (504 h), 22 (528 h) and > 22 days for the activation times of 5, 6, 7, 8, 9 and 10 s, respectively (Fig. S6).

Considering the validity of fifteen days of the samples of cobia fish specimen, the best condition of activation of TTIs was 6 s of UV light. With this load, the smart indicator showed a similar discoloration to the rate of deterioration of the analysed product. Complementing these results, the Arrhenius approach gave an accurate characterisation of the kinetic behaviour of the TTI.

Figure S7 shows the discoloration process of the TTI (activated by 6s of UV light) at various constant temperatures.

The colour change of the label was significantly accelerated during storage at high temperatures (Fig. S7), according to Coelho (2006) and Crano & Guglielmetti (2002), which characterises the operating

Table 2 Images and mean values of chroma b* values of the brand of photochromic labels loaded at different times of activation (5, 6, 7, 8, 9 and 10 s of UV light)

	Activation time (s)					
Crhoma b*	5	6	7	8	9	10
	-27.1 ± 0.26^{a}	-26.9 ± 0.32^{a}	-27.1 ± 0.30^{a}	-27.3 ± 0.29^{a}	$-26.9 \pm 0{,}40^{a}$	-27.5 ± 0.31^{a}
						

Mean values marked with the same letters within the same row did not differ significantly ($P > 0.05$) by Tukey's test.

principle of the active compounds present in TTIs. The colour response of labels obtained good reproducibility during all storage conditions (1, 7 and 10 °C), with low deviations between the values of triplicate measurements.

Table 3 gives an overview of the different kinetic parameters of the Arrhenius plot and the fit to the data.

According to studies by Wanihsuksombat *et al.* (2010), Tsironi *et al.* (2008) and Taoukis & Labuza (1989), smart indicators may be applied when the difference in Ea between them and the food is below 10 kcal mol^{-1}. Thus, evaluating the value above with the data shown in Table 3, it can be concluded that the results of this study are positive to the application of the smart indicator on whole cobia, because the change in quality index (Ea) between TTI and the traditional analysis was successfully validated.

The *OnVu*® TTI B_1 indicator activated by 6 s of UV light showed a discoloration similar to the rate of deterioration of the product analysed, thus proving to be a label of dynamic shelf life that can easily, cheaply and accurately assure consumers of the ultimate point of the quality of the entire cobia, and so becoming a trusted and innovative tool to monitor the supply chain in ice of this product.

It is also emphasised that, due to the possibility to define the shelf life of a TTI by varying the charging times, the label can be used to control the quality during the cold chain of different food products. Because of their low cost, TTIs are also very attractive for use in single packages and product chains with a very low profit margin. These factors lead to high usage flexibility. Implementations of photochromic TTIs combined with effective information management systems will strongly support cold chain management in food supply chains what results in prevention and cost reduction.

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Table 3 Kinetic parameter of the *OnVu*® TTI B_1 label and traditional analysis of the cobia specimen

Analyses	Ea (kcal mol^{-1})	R^2*	Source
TTI activated by 6 s of UV light	37.52	0.989	This work Adapted from Gonzaga (2014)
Microbiological of the cobia	36.81	0.890	
Sensory of the cobia	35.48	0.974	

*Adjustment of data.

Note

[1] Only one activation condition was evaluated, the one selected from the results of the item '*Estimation of the shelf life of cobia during simulation of storage on ice*'.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. TTI colour defined as the end of the shelf-life of the indicator, chroma b* = –7.0. Source: Adapted from Brizio & Prentice (2014).

Figure S2. Average score of sensory attributes relating to: (A) skin (colour/appearance, mucus and odour); (B) eyes (cornea, pupil and form); (C) gills (colour, odour, form and mucus); (D) elasticity of the abdominal muscles, obtained from analysis of whole cobia fish during simulation of storage on ice.

Figure S3. Images of the longitudinal sections of muscle tissue of cobia during simulation of storage on ice for: (a) one day; (b) 7 days; (c) 14 days; (d) 21 days where the bars represent a distance of 100 μm; f = fibre is = intercellular space; cf = crack fibre.

Figure S4. Results of microbiological analyses for Psychrotrophics; *Pseudomonas* spp.; Enterobacteriaceae; H_2S-producing bacteria, done from muscle samples of cobia stored on ice.

Figure S5. Reproducibility of the load process of TTIs (5, 6, 7, 8, 9 and 10s of UV light), evaluated by the mean deviation of chroma b* values.

Figure S6. Discoloration of TTIs (activation conditions of 5, 6, 7, 8, 9 and 10s of UV light) during storage in ice. where chroma b* = –7.0 represents the colour defined as the end of shelf life of the TTI. The error bars represent the standard deviation of triplicates of each measurement.

Figure S7. Response of TTIs (activation conditions 6s of UV light) stored at different temperatures where chroma b* = –7.0 represents the colour defined as the end of shelf life of the TTI. The error bars represent the standard deviation of triplicates of each measurement.