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Developing suitable smart TTI labels to match specific monitoring requirements: The case of *Vibrio* spp. growth during transportation of oysters

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ABSTRACT

Time-temperature control is a critical issue in food logistics and the effective tracking of cold chain conditions is one of the main points to be addressed. There is a need for development and standardization of labels with a flexible range of temperature sensitivity, expressed by activation energy and of response time. Time Temperature Integrators (TTI) are inexpensive, active smart labels that show the time-temperature history of the food product to which they are attached. Enzymatic TTIs are based on a pH decrease resulting from the temperature-dependent enzymatic hydrolysis of a lipid substrate. The colour change of a pH indicator in the solution can be visually assessed or measured instrumentally. The considerations that allow for targeted design of the response characteristics of this type of TTI are the concentration of the enzyme and choice of substrate. This study applied predictive models for growth of *Vibrio parahaemolyticus* (VP) and *Vibrio vulnificus* (VV) growth in oysters and used the information from that work to design enzymatic TTI smart labels with response kinetics suitable for indicating the growth potential of *Vibrio* spp. during the transportation of oysters from harvest to storage for further distribution and sale. The results of the study indicated that the VV- and VP-TTIs developed in this study may be an effective and cost-effective tool for validating improved handling and cooling procedures and for monitoring the transport of oysters.

1. Introduction

Effective control of the chilled distribution of food products is vital to their commercial viability. A substantial portion of chilled products are exposed during distribution to temperatures that deviate significantly from the recommended range. Application of an optimized quality and safety assurance system for product distribution requires continuous monitoring of storage conditions from production to consumption (Tsironi, Gogou, Velliou, & Taoukis, 2008; 2011).

Smart packaging systems can provide information on the quality of food, which may be either indirect (e.g. time-temperature integrators) or direct (e.g. freshness indicators) (Smolander, 2008). Time Temperature Integrators (TTI) are user-friendly and cost-effective tools that can show “an easily measurable, time-temperature dependent change” (Taoukis & Labuza, 1989) that “cumulatively reflects the time-temperature history of the food product” (Gannakourou, Koutsoumanis, Nychas, & Taoukis, 2005). A TTI-based system could lead to realistic control of the cold chain, optimization of stock rotation, waste reduction and more efficient management of product quality. A prerequisite for TTI application is the systematic kinetic modelling of the temperature dependence of the target food products’ quality and shelf-life. Similar kinetic study is needed for the TTI response. By using reliable models of quality deterioration and the kinetics of both product and TTI response, the effect of temperature can be monitored and quantitatively translated to food quality during the life span of the product.

The current TTI technology and the scientific approach with regards to quantitative study of safety risk in foods allow the undertaking of the next important step, i.e. the application of TTIs to manage safety risks of foods (Koutsoumanis & Gougouli, 2015).

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Although the applicability of different TTI systems for monitoring food product quality and shelf-life, mainly of chilled products, has been evaluated in several studies (e.g. Smolander, Alakomi, Ritvanen, Vainionpää, & Ahvenainen, 2004; Tsironi, Stamatiou, Giannoglou, Velliou, & Taoukis, 2011, 2008;; Vaikousi, Biliaderis, & Koutsoumanis, 2009; Xiaoshuan, Gege, Xinqing, Yuanrui, & Xiaoping, 2016), limited information is available for the development of a TTI based food safety management system. Koutsoumanis, Taoukis, and Nychas (2005) introduced a TTI based chill chain management policy, coded Safety Monitoring and Assurance System (SMAS), for the optimization of the distribution of chilled food products within the chill chain. The SMAS led in significantly reduced risk of listeriosis compared to the conventional First-In-First-Out approach. Ellouze and Augustin (2010) developed a microbial TTI for monitoring the growth of *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus* in ground beef and cooked chicken. A recent study by Koutsoumanis and Gougouli (2015) evaluated the applicability of a microbial TTI for monitoring *Listeria monocytogenes* growth in meat pate.

Vibrio spp. are gram-negative, rod-shaped bacteria that occur naturally in estuarine or marine environments. *Vibrio vulnificus* and *Vibrio parahaemolyticus* are the most common *Vibrio* species associated with illnesses resulting from consumption of raw or partially cooked seafood worldwide. After harvest of molluscan shellfish these bacteria grow quickly as ambient temperature rises. This can result in significant numbers especially during the summer harvest, thus posing a health risk if oysters with high numbers of *Vibrio spp.* are ingested raw. Vibriosis is characterized by diarrhea, primary septicemia, wound infections or other extra intestinal infections (Austin, 2010). It has been reported that infections caused by pathogens commonly transmitted through food have declined or are approaching targeted national levels with the exception of *Vibrio* infections.

From 1989 to 2002, the U.S. FDA recorded 341 serious illnesses associated with consumption of raw shellfish containing *V. vulnificus* bacteria. 98% of these cases were from consuming raw oysters. 179 people, over 52%, died from their illness (UC Food safety bulletin). The Centers for Disease Control and Prevention (CDC) started monitoring *V. parahaemolyticus* in coastal waters of USA in May 2013, in 13 states, with federal and state partners. By the end of September 2013 there were 104 cases of vibriosis reported from consuming raw or undercooked shellfish, primarily oysters (CDC, 2013). For the period 2006–2014, among 208 speciated *Vibrio* isolates in USA (96% of the total cases) 131 (63%) were *V. parahaemolyticus*, 27 (13%) were *Vibrio alginolyticus* and 19 (9%) were *V. vulnificus* (CDC., 2015).

In response to the *Vibrio* risk assessment the US Food and Drug Administration (FDA) implemented guidance regarding post-harvest processing (PHP) of Gulf Coast oysters harvested during the summer months (FDA, 2009). According to FAO/WHO (2011) *V. parahaemolyticus* contamination of oysters may reach 60% in seasons of high prevalence. FDA/EPA established a guidance level of 4 log cfu g^{-1} for *V. parahaemolyticus* in ready to eat fishery products (minimal cooking by consumer) (Compliance Program 7303.842).

Growth of *Vibrio spp.* in shellfish after harvest is a typical time-temperature relationship (FDA, 2005) which can be used as a predictive model for growth. TTI developer SME (VITSAB International AB, Sweden) has initiated a development program for suitable TTI-formulations and has proposed four enzyme-substrate formulations with the aim to monitor *V. parahaemolyticus and V. vulnificus* growth. Several investigators have reported the incidence of *Vibrio* spp. on the USA Gulf, Atlantic and Pacific coasts, observing that the highest concentrations occurred during the warmer months of April through October and have evaluated the effect of time and temperature on *V. parahaemolyticus* and *V. vulnificus* growth in oysters or broth systems (Cook, 1994; Gooch, Depaola, Bowers, & Marshall, 2002; Parveen et al., 2013).

The objective of this study was the development of a TTI-based cold chain management system for safety monitoring of oysters at harvest and the evaluation of its applicability for monitoring the risk of growth of *Vibrio* spp.

2. Materials and methods

2.1. Mathematical modelling of V. parahaemolyticus and V. vulnificus growth in oysters

Growth data for *V. parahaemolyticus* and *V. vulnificus* growth in oysters were obtained from literature and analyzed. The Baranyi growth model was used for the prediction of microbial population and used to construct growth curves at specific storage temperatures (Baranyi & Roberts, 1995). Temperature dependence of the growth rate constant, k, was modelled by the Arrhenius equation (Eq. (1)).

$$ln(k) = ln(k_{ref})\text{-}\left(\frac{E_a}{R}\right)\left[\frac{1}{T} - \frac{1}{T_{ref}}\right] \quad (1)$$

where k_{ref} is the growth rate at a reference temperature T_{ref} (in this study 15 °C), T is the temperature in K, E_a (kJ/mol·K) is the activation energy of *Vibrio* spp. growth rates and R is the universal gas constant. The activation energy (E_a) values were estimated from the slope of Arrhenius plots of lnk vs ($1/T\text{-}1/T_{ref}$) by linear regression (Taoukis, Labuza, & Saguy, 1997).

2.2. Time temperature integrators modelling and application

The enzymatic indicator function is based on a colour change in response to a pH decrease caused by controlled enzymatic hydrolysis of a lipid substrate. For example, the colour change of the LP-type enzymatic TTI (VITSAB, Malmo, Sweden) is the result of a controlled enzymatic hydrolysis by a microbial lipase (*Rhizopus oryzae* lipase) of a lipid substrate (a mixture of trilaurin and tripalmitin), whereas in the S-type enzymatic TTI (VITSAB, Malmo, Sweden) the lipid substrate consists of 75% methylstearate and 25% trilaurine. To activate the TTI the enzyme and substrate are mixed by mechanically breaking a separating barrier within the device. The TTI is initially green in colour, becomes progressively yellow/orange and reaches a final red colour at the end of its range. Different combinations of enzyme-substrate and their concentration can be used to provide a variety of response lives and temperature dependencies. A scale depicting the colour changes facilitates visual recognition and evaluation of the magnitude and significance of the colour change observed. The continuous colour change can also be measured instrumentally and the results can be used in a shelf-life management scheme.

In the present study the colour change of the TTI was measured using the Eyeone Pro (X-Rite, Michigan, USA) at D50 illumination and 2° observation angle conditions. The TTI response was modelled by defining a mathematical function that better describes the response vs time at all temperatures. Kinetic modelling of TTI response was based on measurements, at appropriate time intervals, of the response of 5 TTI labels isothermally stored in high-precision (±0.2 °C) low-temperature incubators (Sanyo MIR 153, Sanyo Electric, Ora-Gun, Gunma, Japan) at 15, 20, 25 and 30 °C. The TTI response was modelled by defining a mathematical function that better describes the response vs time at all temperatures.

The enzymatic TTI response change was described by the normalized value (a+b) of the CIELAB scale (Eq. (2))