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Time–Temperature Integrators (TTI)

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Temperature Monitoring in the Cold Chain

It is very important that the shelf life of every existing food should be monitored on a regular basis and in a way that is compatible with the shelf life in question, production volume and environmental conditions to which the food is exposed (or even abused) up to the point of consumption (Fu and Labuza, 1997). The current philosophy for food quality optimization is to introduce temperature monitoring in an integrated, structured quality assurance system, through the entire lifecycle of the product. Ideally, what would be needed is a cost-effective way to either maintain temperature or to individually monitor the temperature conditions of foods throughout distribution in order to indicate their real quality state (Taoukis, 2010). If either one is achieved, it could lead to an effective quality control of the distribution, optimized stock rotation and reduction of waste, as well as give meaningful information on the remaining shelf life of the food. This calls for control over pallets, control over the cartons of food and, finally, over the individual packages at every step in the distribution. Continuous monitoring and verification of the shelf life of foods is necessary and requires the development of practical systems that can monitor, record and translate the temperature effect of food quality from production to consumption. According to the Regulation (EU) No 1169/2011, "*date of minimum durability of a food means the date until which the food retains its specific properties when properly stored*". The food business operators are responsible for shelf life determination for a specific food under defined storage conditions in the temperature range of the real cold chain (Tsironi et al., 2015a).

It has been reported that a substantial portion of chilled and frozen foods are exposed, throughout their distribution, including retail and domestic storage, to effective temperatures that deviate significantly from the recommended range (Fu and Labuza, 1997). Temperature data from recent surveys showed that, despite the good practices and monitoring and control efforts, significant temperature fluctuations are observed during distribution, retail and domestic storage. Within FRISBEE, a Food Refrigeration Innovation for Cold Chain research IP European project, a web-based platform (at http://www.frisbee-project.eu/coldchaindb.html/) has been built for data collection. Data from industry, cold chain parties (distributors, retailers) and consumer surveys, including all stages of the cold chain (from production to consumption), were collected (**Fig. 1**). More than 14.000 actual t–T profiles have been contributed to the database (Gogou et al., 2015; Taoukis et al., 2016).

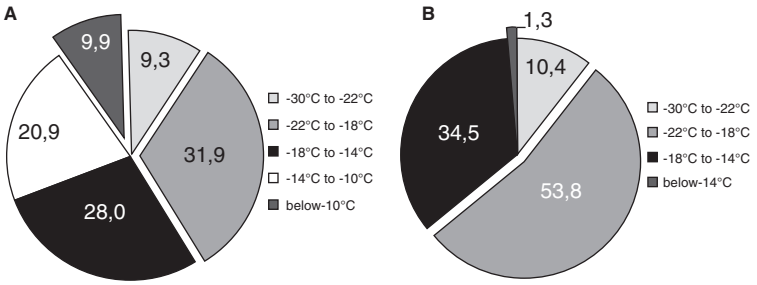


Figure 1 Temperature distribution in: (A) domestic freezers and (B) retail display. Source: Cold Chain Database (2013), FRISBEE project Taoukis et al. (2016).

Quality changes in frozen foods, albeit relatively slow, are very dependent on the storage temperature. For example, 50% vitamin C degradation in frozen green vegetables occurs in 153 days at −20 °C for spinach, but in just 8 days at −5 °C, while the respective values for green beans are 311 and 21 days (Giannakourou and Taoukis, 2003). On the other hand, perishable foods such as chilled fish, show significant sensitivity in temperature deviations. For example, the shelf life of gilthead seabream fillets is 11 days if properly stored at 0 °C and only 4 days or even less at 10 °C (Tsironi et al., 2015b). It becomes evident that temperature monitoring and control within the cold chain is a prerequisite for effective quality management and optimization.

Application of an optimized quality and safety assurance system for the chilled and frozen distribution of foods requires continuous monitoring and control of storage conditions, from production to consumption. Smart packaging systems can provide meaningful information on food quality, either indirectly (e.g. Time–Temperature Integrators) or directly (e.g. freshness indicators) (Smolander, 2008). Time–Temperature Integrators (TTI) are inexpensive, active "smart labels" that can show an easily measurable, time–temperature dependent change that reflects the temperature history of a food to which it is attached (Taoukis and Labuza, 2003). Their change is based on a physical, enzymatic, chemical or microbiological reaction and is expressed by an irreversible color change or a color movement along a scale. The extent to which this change occurs is related to the duration and amount of temperature increase. The visible reaction of the TTI can thus indicate the time–temperature history of the food, changing faster at higher temperatures (Tsironi et al., 2015a).

History of Time–Temperature Integrators and Current Developments

The first application of a "device" to indicate handling abuse dates from World War II, when the US Army Quartermaster Corps used an ice cube placed inside each case of frozen food. The disappearance of the cube indicated mishandling (Schoen and Byrne, 1972). The first patented indicator goes back to 1933 (Midgley, 1933), and over a hundred US and international patents relevant to TTIs have been issued since. During the last 40 years numerous TTI systems have been proposed, of which only a few have reached the prototype and even fewer have reached the market stage. Byrne (1976), Taoukis et al. (1991a, b), Taoukis (2001, 2010) provided overviews of the evolution of TTIs and compiled a list of patents issued up to that time. The first commercially available TTI was developed by Honeywell Corp. (Minneapolis, MN) and was described in detail by Renier et al. (1962). In the early 1970s, the US government considered mandating the use of TTIs on certain products (OTA, 1979). Several companies had proposed temperature indicators (Byrne, 1976; Kramer and Farquhar, 1976) but very little commercial application of the TTI was achieved. Activity in the area of TTI subsided temporarily, as evidenced by a decrease in the relevant publications and in the new TTI models introduced. However, the more sound systems remained available and development continued aiming towards fine tuning of their characteristics and making them more consistent with their claimed performance.

The principle of TTI operation is a mechanical, chemical, electrochemical, enzymatic or microbiological irreversible change usually expressed as a visible response, in the form of a mechanical deformation, color development or movement (Taoukis and Tsironi, 2016). Commercially available TTIs are based on various reaction mechanisms (polymerization, photochromic reactions and diffusion or enzyme reactions) (Taoukis, 2010). They are usually applied in the form of labels on the packaging and thereby act as an indicator.

The ideal TTI should be applicable to the targeted food, practical as a shelf life management tool and cost effective (Taoukis and Labuza, 2003). Such a TTI shall:

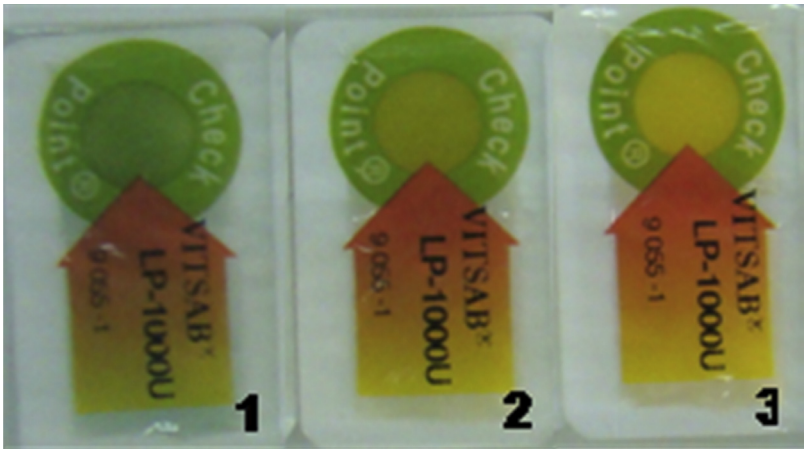
- Be based on a continuous time–temperature dependent change that is expressed in a response that is easily measurable and irreversible.
- Have a response rate that mimics or can be correlated to the food's extent of quality deterioration and residual shelf life.
- Be reliable and reproducible giving consistent responses when exposed to the same temperature conditions.
- Have low cost.
- Be flexible, adaptable to various temperature ranges (e.g., frozen, refrigerated, room temperature), with adjustable temperature sensitivity and useful response periods of a few days up to several months.
- Be small, easily integratable as part of the food package and compatible with a high-speed packaging process.
- Have a long shelf life before activation for use and be easily activatable.
- Be unaffected by ambient conditions other than temperature, such as light, RH and air pollutants.
- Be resistant to normal mechanical abuses with response not alterable by mishandling or tampering.
- Be nontoxic, posing no safety concern in the unlikely situation of food contact
- Be able to transmit in a simple, clear way the intended message to its target, be that distribution handlers or inspectors, retail store personnel or consumers.
- Have a response both visually understandable and adaptable to measurement by electronic equipment for easier and faster information, storage and subsequent use.

Systems that are currently available and strive to comply with these requirements and based on different operational principles are the following:

The CheckPoint® TTI (VITSAB A.B., Malmö, Sweden, www.vitsab.com) is an enzymatic system, which is based on a color change when the pH decreases due to a controlled enzymatic hydrolysis of a lipid substrate by a microbial lipase. For example, the color change of the LP-type enzymatic TTI is the result of a controlled enzymatic hydrolysis by a *Rhizopus oryzae* lipase of a mixture of

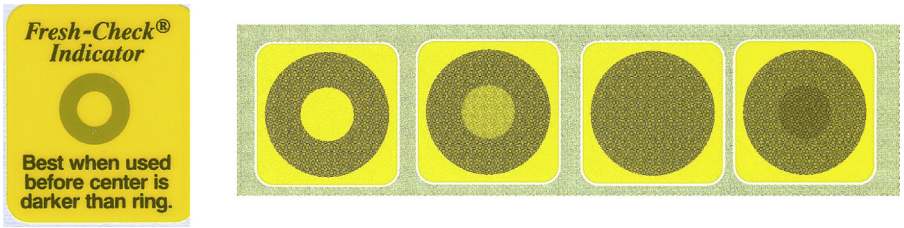
trilaurin and tripalmitin, whereas in the M-type enzymatic TTI the lipid substrate consists of methylmyristate (Pictures 1 and 2). To activate the TTI the enzyme and substrate are mixed by mechanically breaking a separating barrier within the device. Two color configurations are possible, depending on the specific pH indicators: a bi-color, in which the TTI changes gradually from deep green to bright yellow, and a tri-color in which the TTI changes gradually from deep green to yellow to red. 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 color changes facilitates visual recognition and evaluation of the magnitude and significance of the color change observed. The continuous color change can also be measured instrumentally and the results can be used in a shelf life management scheme (Tsironi et al., 2017a,b). VITSAB has developed an enzymatic TTI for application on vacuum- or modified atmosphere (MA) packaged fresh seafood imported to the USA by several importing companies. The import of these products is covered by FDA's Import Alert #16–125 (26/01/2018, https://www.accessdata.fda.gov/cms_ia/importalert_28.html).

The Fresh-Check® TTI (Temptime Corp., NJ, USA, http://www.fresh-check.com/) (successor to Fresh-Check of Lifelines) is based on a solid state polymerization reaction (**Picture 3**). The TTI function is based on the property of disubstituted diacetylene crystals to polymerize through a lattice-controlled solid state reaction, resulting in a highly colored polymer. The response of the TTI is the



Picture 1 Visualization of the color change of bi-color Vitsab LP-type TTI.

Picture 2 Visualization of the color change of tri-color Vitsab M-type TTI.



Picture 3 Visualization of the color change of Fresh-Check® TTI.

color change as measured in terms of a decrease in reflectance. The color of the "active" center of the TTI is compared to the reference color of a surrounding ring. Before using the indicators, which are active from the time of their production, the TTIs must be stored at very low freezer temperatures, where change is very slow.

The OnVu™ TTI (B1 OnVu™, Bizerba, Germany, www.bizerba.com) is a solid state reaction-based TTI, which is based on the inherent reproducibility of reactions in the crystal phase (Patent EP 1049930 B1). Photosensitive compounds, such as spiropyrans, are excited and colored by exposure to low wavelength light. This colored state (dark blue) reverses to the initial colorless state at a temperature dependent rate, as illustrated in **Picture 4** (Kreyenschmidt et al., 2010). This temperature dependence results in a very long response at low freezing and a much shorter at near sub-freezing and zero temperature ranges. The determining factor for this TTI is the time of activation; the higher the charging time, the longer the shelf life of the TTI. By controlling the type of photochromic compound and the length of UV light exposure during activation the length and the temperature sensitivity of the TTI can be set (Tsironi et al., 2011).

The TOPCRYO (former eO®) TTI (CRYOLOG, Gentilly, France, www.cryolog.com) is based on a time- and temperature-dependent pH change, caused by controlled microbial growth occurring in the gel, that is expressed as color changes using suitable pH indicators, as indicated in **Picture 5** (Louvet et al., 2005; Ellouze et al., 2008). The response of the TTI is claimed to mimic microbiological spoilage of the monitored food, as its response is based on the growth characteristics of similar microorganisms, such as select patented strains of the microorganisms *Carnobacterium piscicola*, *Lactobacillus fuchuensis* and *Leuconostoc mesenteroides*. The pH drop occurs with a color change of the pH indicator from green to red.

The TT Sensor™ TTI (CCL Design, Strongville, OH, USA, http://www.ccl-design.de) is based on a diffusion-reaction concept. A polar compound diffuses between two polymer layers and the change in concentration causes the color change of a fluorescent indicator from yellow to bright pink.

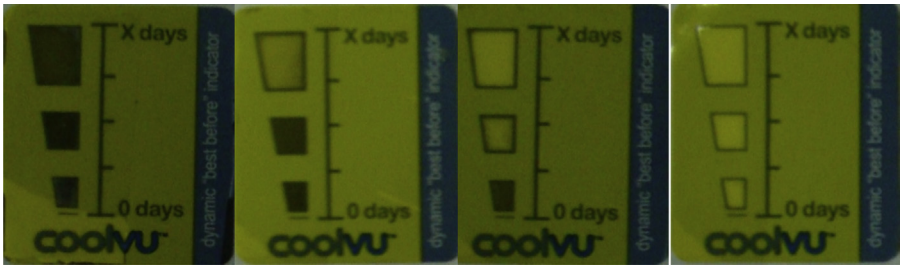
The 3 M Monitor Mark® (3 M Co., St. Paul, MN, USA, www.3m.com) is based on diffusion of proprietary polymer materials. A viscoelastic material migrates into a light-reflective porous matrix at a temperature-dependent rate. This causes a progressive change in the light transmissivity of the porous matrix and provides a visual response. The TTI is activated by adhesion of the two materials that, before use, can be stored separately for a long period at ambient temperature.

The CoolVu metal etching Al-TTI system (Freshpoint, Nesher Haifa, Israel, www.freshpoint-tti.com/) is assembled from a metal (aluminum) base label and a secondary transparent label that contains an etchant (**Picture 6**). Once the etchant label is placed on top of the metal layer, the label is activated. The etching process is time and temperature dependent, and creates a visual change at the end of the process. By changing the concentration of the glue or the thickness of the aluminum layer the label can be adapted to different products with different kinetics.

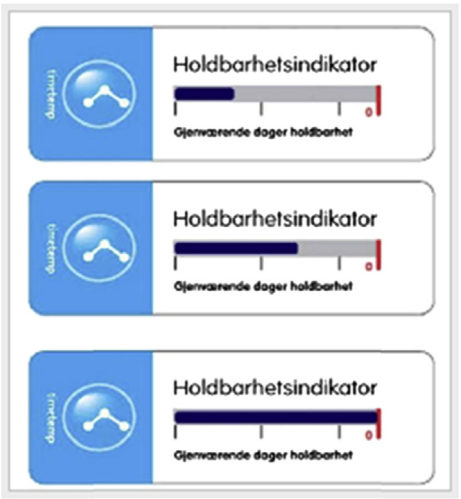
The Keep-it® indicator (Keep-it Technologies® AS, Oslo, Norway, www.keep-it.com/) is based on a time- and temperature-dependent migration of a pH modifying agent into a mutarotational reducing system, as indicated in **Picture 7** (Taoukis and Tsironi, 2016).

Picture 4 Visualization of the colour change of OnVu™ TTI.

Picture 5 Visualization of the color change of CRYOLOG TTI.



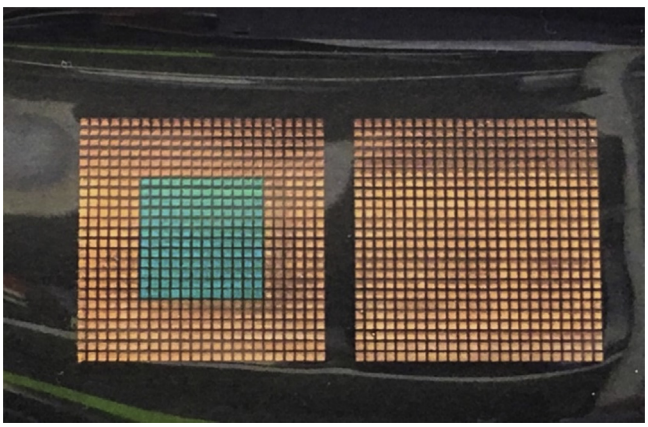
Picture 6 Visualization of the response of the CoolVu TTI.



Picture 7 Visualization of the response of the Keep-it TTI. Source: www.keep-it.no.

A novel TTI has been recently developed by FreshStrips (FreshStrips B.V., Eindhoven, Netherlands, www.freshstrips.co), based on shape memory of a mechanically embossed chiral nematic (molecules aligned in loose parallel lines) polymer network of liquid crystals, as indicated in **Picture 8** (Davies et al., 2013).

Several TTI developed at the laboratory scale and still in early research stage have been reported (**Table 1**). Some represent true innovation and many are based on limited scope application of systems as trivial as enzymatic or non-enzymatic browning or growth of food related bacteria.



Picture 8 Visualization of the response of the FreshStrips TTI.

Table 1 Recent progress in design and development of Time-Temperature Indicators

TTI type/sensing element	*Application*	*Reference*
Isopropyl palmitate	Angelica juice	Kim et al., 2016
Chitosan/PVA film with Anthocyanin from red cabbage	Milk	Pereira et al., 2015
Glycerol tributyrate - *Aspergillus niger* lipase	–	Wu et al., 2015
Tyrosinase	–	Kocak and Soysal, 2014
Lactic acid bacteria loaded onto Ca-alginate microparticles	Beef products	Choi et al., 2014
PEGylated laccase	Kimchi	Kang et al., 2014
Polydiacetylene -SiO_2 - surfactant	–	Nopwinyuwong et al., 2014
Weissella cibaria - Man-Rogosa-Sharpe broth	Chicken breast	Park et al., 2013a
Laccase-sodium azide	–	Park et al., 2013b
Ag shell - Au nanorod	–	Zhang et al., 2013
Alkaline lipase - PVA	Milk	Lu et al., 2013
Phenol red - Carbamide - Urease	–	Wu et al., 2013
Laccase (*Pleurotus ostreatus*) – PEGylated laccase (*Trametes versicolor*)		Kim et al., 2013
PDA - Silica nanocomposites	–	Nopwinyuwong et al., 2012
Weissella cibaria	Ground beef	Kim et al., 2012a
TOPAS 5013 - BBS chromophores	High pressure treated foods	Lee and Shin, 2012
Burkholderia cepacia lipase	Ground beef	Kim et al., 2012b
Gelatin-templated gold nanoparticles	Frozen foods	Lim et al., 2012
Lactic acid bacteria	–	Kim et al., 2012c
Lactic acid bacteria	Minced meat	Vaikousi et al., 2009

Methodology for Appropriate TTI Selection and Application

A prerequisite for application of TTIs is the systematic kinetic modelling of the temperature dependence of shelf life of the target food. Similar kinetic study is needed for the TTI response. Based on reliable models of the shelf life and kinetics both of the food deterioration and the TTI response, the effect of temperature can be monitored, and quantitatively translated to food quality, from production to the point of consumption (Taoukis and Labuza, 1989a, b; Taoukis, 2001). Most of the studies have been conducted on chilled food, mainly meat and fish products under isothermal and dynamic temperature conditions simulating real cold chain scenarios (Mai et al., 2011; Smolander et al., 2004; Tsironi et al., 2008, 2011, 2017a,b; Raab et al., 2011; Vaikousi et al., 2009; Xiaoshuan et al., 2016). A limited number of studies evaluated the applicability of specific commercial TTIs (mainly enzymatic and photochromic) for monitoring quality and shelf life of frozen meat or fish (Giannoglou et al., 2014; Han et al., 2012; Tsironi et al., 2016).

The basic principles of TTI modelling and application for quality monitoring have been established by Taoukis and Labuza (1989a,b). Loss of shelf life of a food (based on the deterioration of the selected index, C) can be expressed (Taoukis et al., 2012):

$$f_q(C) = k_{C_{ref}} \exp\left(-\frac{E_A}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) t \quad (1)$$

where E_A is the activation energy of the reaction that controls quality loss, T is the absolute temperature, R is the universal gas constant, t is time and k is the rate.

In a similar manner to **Eq. (1)**, a response function F(X) can be defined for a TTI such that $F(X) = k_I t$, with k_I an Arrhenius function of T.

To represent the integrated effect of the temperature variability on food quality degradation, the term "effective temperature", T_{eff}, is used. T_{eff} is defined as the constant temperature that results in the same quality value as the variable temperature distribution over the same time period. This approach equals the overall effect of a non-isothermal handling with a single, constant value.

For a TTI exposed to the same temperature fluctuations, T(t), as the food, and corresponding to an effective temperature T_{eff}, the response function can be similarly expressed as:

$$F(X) = k_{I_{ref}} \int_0^t \exp\left(-\frac{E_{A_I}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) dt = k_{I_{ref}} \exp\left(-\frac{E_{A_I}}{R}\left(\frac{1}{T_{eff}} - \frac{1}{T_{ref}}\right)\right) t \quad (2)$$

where $k_{I_{ref}}$ and E_{A_I} are the Arrhenius parameters of the TTI.

Thus, the basic elements for a TTI-based food quality monitoring scheme are (a) a well-established kinetic model to describe quality loss of the food, (b) the response function of the TTI and (c) the temperature dependence of both food quality loss and TTI response rate, expressed by the respective values of the activation energies. The essence of the TTI implementation algorithm lies in the calculation of the T_{eff} of the exposure (**Eq. 2**), based on the TTI response reading that is assumed to describe the integrated

effect of temperature history on food quality loss (Fig. 2). This assumption requires that food quality degradation and TTI response rate are similarly affected by temperature, i.e. the activation energies of the two phenomena do not differ by more than 25 kJ/mol. Under these conditions, the application scheme would reliably provide the extent of the quality deterioration of the food and a prediction of the remaining shelf life at any assumed average storage temperature (Taoukis et al., 2012, 2014).

TTI Response Study

A sufficient amount of TTI labels are obtained to be thoroughly studied at a minimum of 3 storage temperatures. The labels are activated according to the guidelines by the TTI provider and are adhered to a glass plate. Glass has high thermal capacity and thus the temperature of the labels during the short time of measurement is not affected. Multiple TTI samples are isothermally stored in controlled incubators, e.g. at −5, −8 and −12 °C for testing at frozen conditions, and 0, 5 and 10 °C for testing at refrigerated conditions. Satisfactory temperature stability will be achieved with commercial freezers or refrigerators, by installing high precision temperature controllers. The temperature profile of each incubator can be recorded during the experiments using low cost, programmable, downloadable data loggers. Testing of the TTIs is based on measurements at appropriate time intervals of the color change of multiple TTI samples (3–5 samples per temperature). This can be implemented using a color measuring instrument, if available. Values of the different color parameters (i.e. L, a, b) are plotted vs time for all temperatures studied and mathematical equations that most effectively describe their changes are selected. Otherwise, visual evaluation of the TTI response changes can be conducted.

To assist this visual reading, color scales can be employed. Multipoint color scales, one for each type of the studied TTI labels, are constructed and used for TTI color evaluation by the panel (Picture 9). For the construction of the color scales, TTI labels are activated and left at T = 25 °C. When a color change is visually observed, the color of the TTI is instrumentally measured and then the label is numbered and placed on a glass surface which is then stored at −30 °C. Thus, each label of the constructed color scales corresponds to a specific measurement of color (i.e. L, a, b). This procedure is repeated for the whole range of color change for each TTI type.

In order to confirm that visual scoring gives results similar to the respective ones obtained by the instrumentally measured color of the labels, when the color of the TTI label is instrumentally measured, panellists are asked to compare the labels' color with the numbered color scale. The L, a, b values estimated by the colorimeter are then compared to the L, a, b values of the label indicated by the panellists. The color scales should be stored at −30 °C and frequently checked to ensure color stability (in case of decolorisation of the TTIs, the color scale must be replaced). The response time (i.e. time from activation to endpoint, as defined by the TTI provider) at each studied temperature is determined (Tsironi et al., 2015a).

Shelf Life Testing of Food

A first estimation of the shelf life of a specific food can be made based on a literature review and past experience. Shelf life testing experiments are designed to determine the shelf life of a food under given conditions and validate previous shelf life estimations. The target foods are produced and packaged under the desired conditions. A sufficient number of packages are stored at a minimum of 3 isothermal conditions in incubators with temperature control, with the higher temperature reaching abuse conditions that have been reported in the frozen or refrigerated food chain (e.g. at −5, −8 and −12 °C for testing at frozen conditions, and 0, 5 and 10 °C for testing at refrigerated conditions). Temperature in these controlled incubators should be constantly monitored with appropriate data loggers. This accelerated shelf life testing (ASLT) will (i) enable the determination of the relatively long shelf lives e.g. of frozen foods in shorter periods and (ii) evaluate the effect of temperature abuse on the shelf life stability of the food. Control samples are

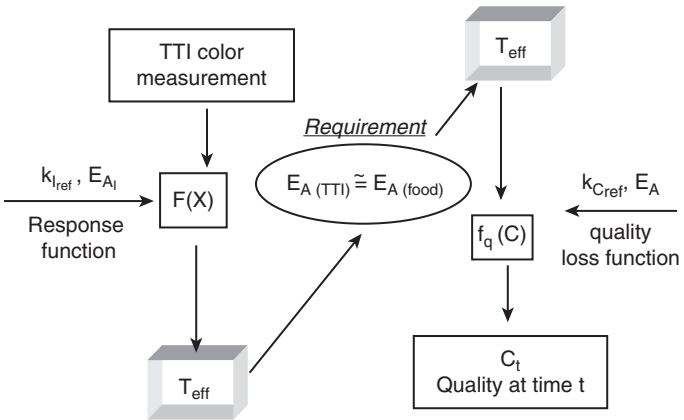
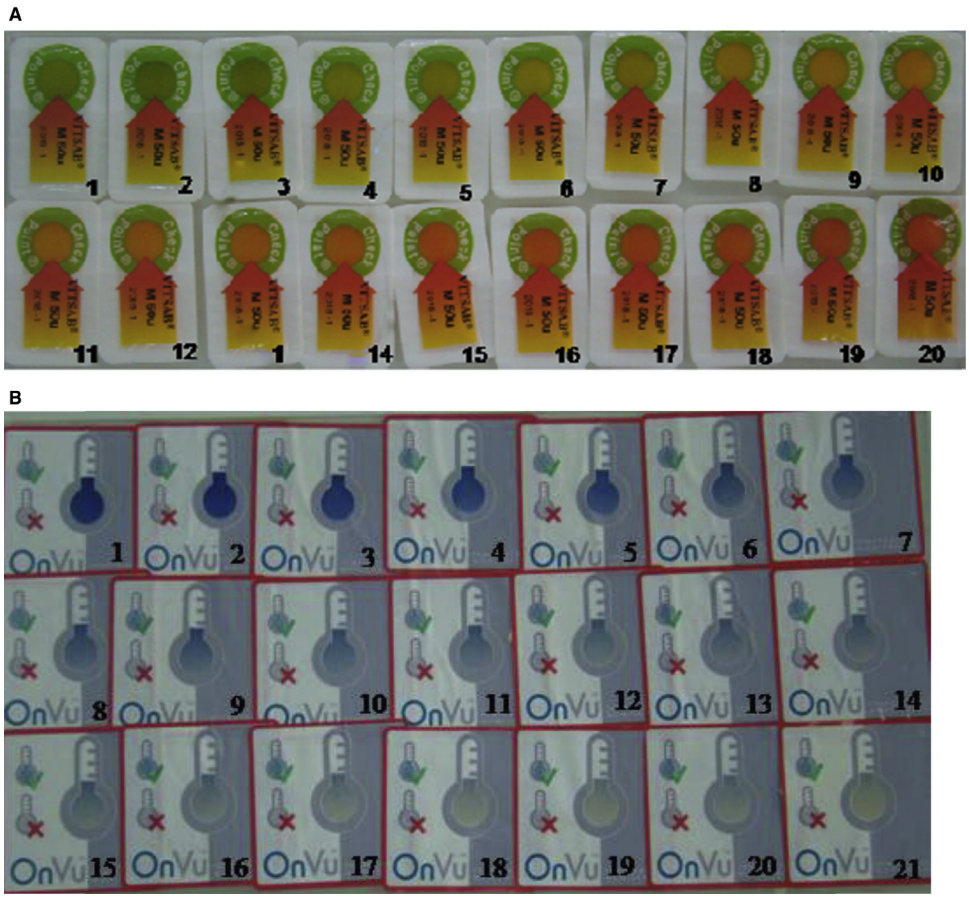


Figure 2 Application scheme of TTI as quality monitors and tools for predicting food remaining shelf life. All kinetic data necessary as input for food quality loss and TTI response are also shown (Taoukis et al., 2012).



Picture 9 (A) M-type enzymatic TTI label color scale, (B) OnVu™ TTI photochromic TTI label color scale.

also kept at recommended conditions (e.g. −18 °C) in order to validate the predictions by the accelerated shelf life experiments. The number of samples and controls required should be based on a detailed experimental design. In general, at least 10 packages per storage condition are required. If a large quantity of the food packages are available, extra samples should be placed into each storage condition (Fu and Labuza, 1997). Samples are taken at appropriate time intervals to allow for efficient kinetic analysis of quality deterioration (Tsironi et al., 2015a).

The criterion for the limits of acceptability for shelf life determination may be variable, based on the quality determining parameters. For example, frozen foods degrade mainly by slow chemical reactions such as loss of nutritional value (e.g. vitamin C content for some frozen fruits and vegetables) or other chemical changes (e.g. development of lipid oxidation resulting in the typical rancid taste) (Tsironi et al., 2015a). On the other hand, microbial activity dominates the spoilage mechanism of foods stored at refrigerated conditions, as for example for perishable meat and fish products. Values of the different measured indices are plotted vs time for all temperatures studied and mathematical equations that most adequately fit the data are selected (Andreou et al., 2018; Giannakourou and Taoukis, 2003; Sofra et al., 2018; Tsironi et al., 2009a,b, 2017a,b). Microbiological, chemical or instrumental measurement that closely correlates to sensory changes can be the criterion for shelf life evaluation.

The Arrhenius equation is often used to describe the temperature dependence of deterioration rate (Taoukis et al., 1997). Thus, by studying a deterioration process and measuring the rate of loss at two or three temperatures (higher than storage temperature), one could then extrapolate on an Arrhenius plot to predict the deterioration rate at the desired storage temperature. This is the basis for ASLT. However, it should be noted that for frozen foods, extrapolation from temperatures above 0 °C are meaningless for shelf life prediction (Fu and Labuza, 1997).

Case Study: Selection of Appropriate Enzymatic TTI for Shelf Life Monitoring of Chilled Gilthead Seabream Fillets

Shelf Life Modelling of Chilled Gilthead Seabream Fillets

The selection and application of appropriate TTI smart labels for monitoring the quality of chilled fish is one of the objectives of the ERA-NET research project SUSHIFISH "SUStainable production of HIgh quality aquaculture FISH using innovative tools and production strategies and integrating novel processing methods and cold chain management", co-funded by the European Union

(European Regional Development Fund - ERDF) and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation 2014–20" of the National Strategic Reference Framework (NSRF) (2016–19). Raw marine cultured gilthead sea bream (*Sparus aurata*) fillets were stored aerobically in controlled temperature cabinets (Sanyo MIR 553, Sanyo Electric Co, Ora-Gun,Gunma, Japan) at constant temperatures (0, 5, 10 and 15 °C). Electronic, programmable, miniature data loggers (COX TRACER®, Belmont, NC) constantly monitored the temperature in the incubators. Quality assessment of fish samples was based on microbiological analysis (total viable count, *Pseudomonas* spp., lactobacilli, *Enterobacteriaceae* spp.), pH, color and texture measurement and sensory scoring. Samples were taken at appropriate time intervals to allow for efficient kinetic analysis of quality deterioration. All determinations were made in duplicate samples.

Pseudomonas spp. dominated spoilage at all temperature conditions for aerobically packed filleted fish. Sensory scores were modelled by apparent zero order lines. The organoleptic deterioration had high correlation with microbial growth. At all temperatures studied, the time for sensory rejection (i.e. score 5 for the overall sensory impression) coincided with a *Pseudomonas* spp. level of 10^6 cfu/g. Temperature dependence of the rates of *Pseudomonas* spp. growth and sensory degradation was adequately described by Arrhenius kinetics in the temperature range studied (i.e. 0 to 15 °C). The activation energies, E_a, were determined as 55.2 and 63.9 kJ/mol for *Pseudomonas* spp. growth and sensory deterioration, respectively ($T_{ref} = 4$ °C) (Tsironi et al., 2009a,b).

Shelf life plots are practical and easier to understand as one can read directly the shelf life of the food at any storage temperature. The shelf life of chilled gilthead seabream fillets at all storage temperatures in the range 0–15 °C is illustrated below (Fig. 3).

Response Kinetics of the Enzymatic TTI

The color change of the M-type enzymatic TTI (M Check Point®, VITSAB, Malmo, Sweden) is the result of a controlled enzymatic hydrolysis by a microbial lipase (*Rhizopus Oryzae* lipase) of a lipid substrate (methylmyristate). This initially green colored TTI progressively turns yellow/orange, finally reaching a red color. Kinetic modeling of TTI response was based on measurements, at appropriate time intervals, of the response of 5 TTI labels isothermally stored in low-temperature, high-precision incubators (Sanyo MIR 553, Sanyo Electric Co, Ora-Gun, Gunma, Japan) from −15 to −5 °C. The color change of the TTI was measured instrumentally using the Eye-one Pro (X-Rite, Michigan, USA) at D50 illumination and 2° observation angle conditions. The TTI response was modeled by defining a mathematical function that better describes the response vs time at all temperatures and initial charging conditions or enzyme concentrations. SYSTAT 10.2® Software was used to calculate the model coefficients by nonlinear regression.

The enzymatic TTI response change was described by the normalized value (a + b) of the CIELAB scale (Eq. 3)

$$\text{norm}\ (a+b) = \frac{(a+b) - (a+b)_{min}}{(a+b)_{max} - (a+b)_{min}} \tag{3}$$

Eq. (3) represents the M-type TTI response ranging from a value of 0 for green to a value of 1 for red. The orange-red hue considered as the visual end point of the TTI corresponds to an instrumental value of 0.8. When plotted as a function of time, response follows a sigmoidal pattern, described by a logistic type equation (Eq. 4)

$$\text{norm}\ (a+b) = \frac{1}{1 + \exp\left(\frac{k_{1I} - t}{k_{2I}}\right)} \tag{4}$$

where k_{1I} and k_{2I} are the response rate constants which are functions of enzyme concentration and storage temperature (Giannoglou et al., 2014; Tsironi et al., 2008). Note that $1/k_{2I}$ is the exponential rate constant i.e. the phase's slope in which the TTI's response changes exponentially with time. At each temperature, values of k_{1I} and k_{2I} were determined by non-linear regression analysis (Sigma Plot 10.0).

Values of k_{1I} and k_{2I} were expressed as a function of enzyme concentration and a composite model was developed which can determine the TTI response of a known enzyme concentration at a selected time–temperature scenario (Eq. 5).

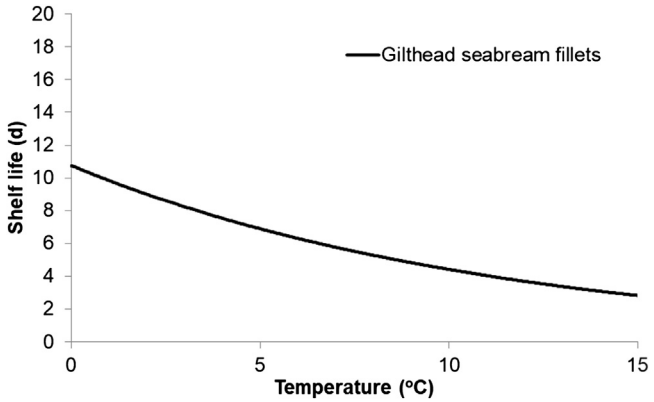


Figure 3 Shelf life (d) curve of chilled gilthead sea bream fillets at the temperature range 0–15 °C.

$$\mathrm{norm}(a+b) = \frac{1}{1+\exp\left(\frac{k_{1,ref}{}^{*}C^{-B_1{}^{*}}\exp\left[\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]-t}{k_{2,ref}{}^{*}C^{-B_2{}^{*}}\exp\left[\frac{E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]}\right)} \quad (5)$$

where T is the storage temperature (K), E_a is the activation energy (E_a = 80.5 kJ/mol), R is the universal gas constant, T_{ref} is a reference temperature (T = 5 °C), C is the enzyme concentration and $B_{1,2}$ are constants ($B_1 = 1.061$, $B_2 = 1.14$), $k_{1ref} = 136.3\ d^{-1}$ and $k_{2ref} = 44.5\ d^{-1}$.

Alternatively, the response time of the enzymatic TTI can be determined using the color scale consisting of TTIs at different levels of color change. End point color is noted by a single value on the scale of the TTI (Fig. 4). For M-type enzymatic TTI label end point value is 12.

Based on the above described methodology, the total response times (time from activation to endpoint) of the studied TTI labels for the different enzyme concentrations and storage temperatures are illustrated below. It is evident that the lower the enzyme concentration or the higher the charging time of the TTI, the longer the shelf life at a given storage temperature.

Selection of Appropriate TTI for Shelf Life Monitoring of Chilled Gilthead Seabream Fillets

In order to select the appropriate TTI, based on the shelf life studies on the target food and the response profiles of the TTI, the shelf life curves of the TTI and the food can be combined to obtain an adequate match between the food shelf life and the response time of the TTI label in the refrigerated range. In practice, this requires that the TTI response kinetics are similar to the kinetics of quality loss of the food, which means similar E_A values. Sometimes this exact matching is not possible, as shown in Fig. 5, where the M-type enzymatic TTI is investigated to monitor the shelf life of chilled gilthead seabream fillets. In this case, a suitable TTI can be selected if it shows satisfactory correlation with the target food in the temperature range of 0 to 2 °C; at lower (recommended) storage temperatures it gives higher response times than the respective shelf life of the food.

Based on the developed mathematical models, M-26U enzymatic label was well correlated with the shelf life of chilled gilthead sea bream fillets (Fig. 5). Based on this selection, if the fish is stored at very low temperatures close to 0 °C, the end of the shelf life will be limited by the expiration date on the fish package. On the other hand, if abuse temperatures prevail, then the TTI will signal poor quality of the product slightly before the end of shelf life.

Application of the Selected TTI for Monitoring Quality and Shelf Life of Gilthead Seabream Fillets in the Real Cold Chain

At any point during distribution, the TTI response can be correlated to the quality level and thus provide a reliable indication of the food remaining shelf life.

In order to demonstrate the applicability of the developed models and the selected enzymatic TTI, a realistic distribution scenario in the current cold chain was simulated. It includes an initial stage of 12 h storage in the processing (filleting) and packing plant, followed by transportation and storage for 1 day and finally retail display for 1 day. Subsequently, fish fillets are purchased by the final consumers and are stored in domestic refrigerators for 2 days before cooking (Fig. 6). The extent of quality deterioration (*Pseudomonas* spp. growth) at the end of each stage of the cold chain and the remaining shelf life at a subsequent constant storage

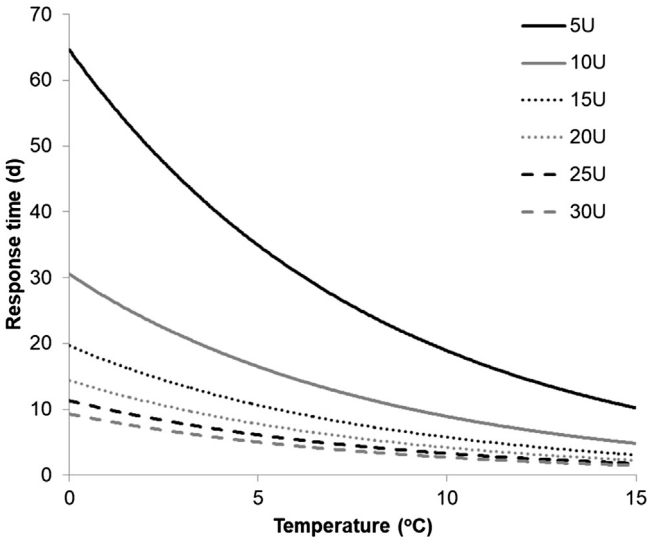


Figure 4 Total response time of M-type enzymatic TTI as a function of temperature and enzyme concentration calculated by the composite model.

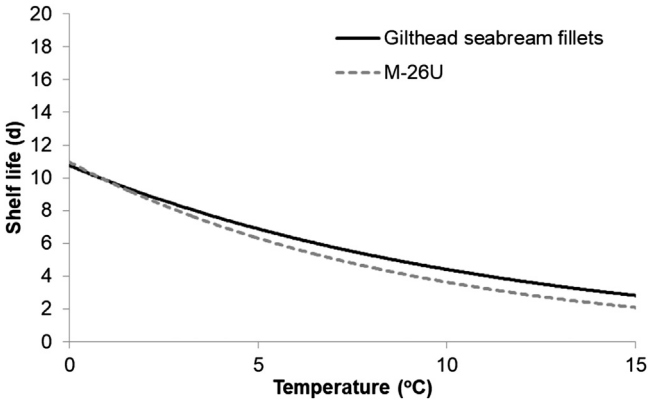


Figure 5 Shelf life (d) of chilled gilthead sea bream fillets at different storage temperatures together with the matching TTI response curve.

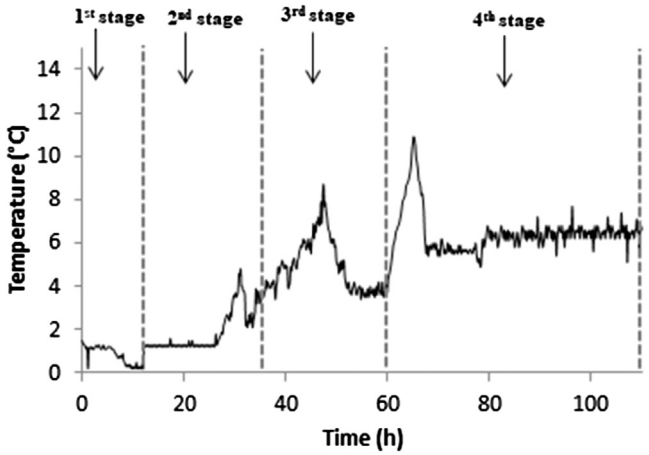


Figure 6 Indicative time–temperature profile of distribution and storage of chilled fish fillets in the real cold chain.

temperature (i.e. 0 °C) was estimated based on (i) the mathematical models developed by Tsironi et al. (2009a,b) ($SL_{R,food}$) and (ii) by the TTI response ($SL_{R,TTI}$) (Fig. 7).

The results of the study indicated the applicability of the selected enzymatic TTI smart labels for monitoring chilled gilthead sea bream quality and shelf life from production to consumption. At any point in the cold chain, the response of the M-26U enzymatic TTI could be correlated with the quality level and provide an indication of the product's remaining shelf life. At the end of the

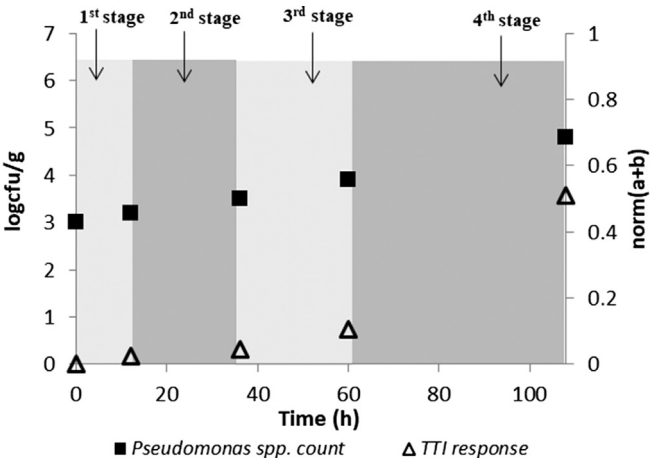


Figure 7 *Pseudomonas* spp. growth and TTI response at the end of each stage of the simulated scenario for distribution and storage of gilthead seabream fillets.

Table 2 Quality deterioration and remaining shelf life of chilled gilthead seabream fillets at the end of each stage of the cold chain (initial *Pseudomonas* spp. Count = 10^3 cfu/g, *Pseudomonas* spp. limit of acceptability = 10^6 cfu/g)

	1st stage $T_{eff,} = 1.0\,^{\circ}C$ *Duration:12 h*	*2nd stage* $T_{eff} = 2.1\,^{\circ}C$ *Duration:24 h*	*3rd stage* $T_{eff} = 5.1\,^{\circ}C$ *Duration:24 h*	*4th stage* $T_{eff} = 6.5\,^{\circ}C$ *Duration:48 h*
N_{Pseudo} (log cfu/g)	3.2	3.5	3.9	4.8
$SL_{R,food}$ (d) at 0 °C	10	9	8	4
TTI response (norm (a + b))	0.0245	0.0450	0.1046	0.5109
$SL_{R,TTI}$ (d) at 0 °C	10	9	7	3

simulated scenario of 108 h, the SL_R based on the nominal "use by" date on the food package, which does not consider the time–temperature history of the products, is 7 days. However, the actual SL_R based on the kinetic models of the microbial spoilage and the T_{eff} indicated by the TTI is 4 d (Table 2).

Conclusions and Future Trends

Cold chain optimization and effective management will continue to be focused upon in research, industrial practices, and regulatory efforts, in the effort to assure that safe, high quality perishable foods are supplied to consumers. Integrated systems, based on the availability of quality data and temperature history of individual product units, will be applied and validated in practice, and TTIs will be combined with smart technologies to enhance the implementation of current traceability requirements dictated by regulation or industry initiatives.

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