

Performance Specification of Time–temperature Integrators Designed to Protect Against Botulism in Refrigerated Fresh Foods

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ABSTRACT: Time–temperature integrators (TTIs) may provide a means to monitor safety of fresh foods packaged in reduced-oxygen environments. Commercially, TTI's have not been fully realized, possibly due to confusion over selection, performance, and reliability. The purpose of this work was to develop a framework for establishing performance targets for TTI's with foods that have potential to cause foodborne botulism. An Arrhenius-type curve was developed that offers safety margins that satisfy regulatory and shelf life requirements. TTI's developed as specified should minimize “false unsafe” readings that lead to destruction of safe product. The approach could be used to establishing guidelines for application of TTI's to food safety verification.

Keywords: TTI, seafood, fish, package, MAP, vacuum, oxygen, botulism

Background

Motivation for reduced-oxygen packaging

Reduced-oxygen packaging (ROP) of fresh foods offers at least 2 major benefits: (1) improved production, handling and distribution efficiency and (2) shelf-life extension. Reduced-oxygen packaging is usually performed with vacuum packaging (VP) or modified atmosphere packaging (MAP). Vacuum packaging involves removal of gas from a package, whereas MAP involves altering the gaseous composition of the atmosphere within a package in a prescribed manner.

Introduction of VP for distribution of chilled beef is recognized as one of the most important developments in meat handling during the 20th century (Robertson 1993). Prior to this innovation, large portions of animal carcasses were transported to local butchering operations. Today, slaughter and gross portioning are performed in well-controlled, centralized facilities. Unused carcass parts are removed prior to distribution. Beef products are now internationally available as standardized, easily handled, vacuum-packaged units known as “boxed beef”.

Once beef is vacuum packaged, the residual gaseous environment becomes deficient in oxygen and enriched in CO₂. This type of environment has been shown to be beneficial for two reasons: (1) growth of aerobic microorganisms responsible for spoilage is reduced and (2) CO₂ dissolves in moist foods and establishes an equilibrium with

carbonic acid, which reduces pH (increase acidity) and further inhibits microbial growth. Ample research has shown that many other types of fresh foods can also benefit from ROP including poultry and seafood, as well as respiring products such as fruits and vegetables.

Safety issues related to reduced-oxygen packaging

Botulism is a serious paralytic disease caused by eating foods that contain a potent nerve toxin produced by the bacterium *Clostridium botulinum*. Historically, botulism was associated with canned foods that were either damaged or insufficiently sterilized. Recent trends toward ROP of fresh foods are creating more avenues of risk for this disease. Reduced-oxygen packaging of fresh or minimally processed seafood may be one of the most significant risks facing industry and consumers since it has been shown that toxin may be present prior to obvious spoilage (FDA 2001). This danger is compounded by the fact that seafood is often cooked less severely than other foods. Therefore, solving safety issues related ROP of fresh seafood may pave the way for application of ROP technologies to many types of foods.

Seafood spoilage

Seafood consumption in the United States has been steadily increasing since the mid-1950s (Gerdes and Valdez 1991). Data from the Florida Department of Environmental Protection-Marine Fisheries values annual landed seafood in Florida at

over \$200 million. Demand for fresh seafood continues to grow, while our ability to preserve product quality remains limited. Although studies have shown that MAP may be capable of extending the shelf-life of fresh fish (Hong and others 1996), its use is limited due to the danger of pathogenic bacteria causing toxicity prior to obvious spoilage. This is due to two diametrically opposed roles of spoilage bacteria. On one hand, it is desired to reduce spoilage organisms to preserve quality. On the other, the Food and Drug Administration (FDA) is concerned that if spoilage organisms are reduced or eliminated, products may become toxic prior to onset of noticeable spoilage.

Spoilage begins as soon as fish die. Normal defense mechanisms seize, and a series of changes caused by bacteria, enzymes and chemical action allow spoilage to begin. Bacteria are believed to be the most important cause of seafood spoilage (Price 1990). In addition to bacteria, oxygen in the atmosphere can attack fats causing rancidity, off-odors and off-flavors. This is especially important in fatty fish such as salmon and mackerel. Evidence of oxidative damage has been noted while attempting to preserve fish on deep-water fishing vessels, where it was found that gills of ice-immersed fish exposed to “air pockets” turned brown, while unexposed gills remained red (Scarlati 1965).

Under traditional conditions, aerobic bacteria tend to be the major cause of spoilage in fish. Therefore, researchers have been attempting to extend the shelf life of

Table 1—Data demonstrating potential for toxigenesis prior or coincident to observable spoilage under mildly abusive temperature conditions

			Temperature (°C)					
			4		8		16	
			Spoilage (d)	Toxic (d)	Spoilage (d)	Toxic (d)	Spoilage (d)	Toxic (d)
Reddy and others 1996	Tilapia	Air	10	>47	6	20	3	4
		75% CO ₂ /25% N ₂	80	>90	17	40	4	4
		Vacuum	47	>90	10	17	3	3
Reddy and others 1997a	Salmon	Air	24 to 27	>66	13 to 17	17	4	4
		75% CO ₂ /25% N ₂	55 to 62	>80	20 to 24	24	5 to 6	4
		Vacuum	34 to 38	>66	6 to 10	10	3	3
Reddy and others 1997b	Catfish	Air	13	>54	6	9	3	3
		75% CO ₂ /25% N ₂	38 to 40	>75	13	18	4	4
		Vacuum	20 to 24	46	6	6	3	3

fresh fish by reducing exposure to oxygen via modified atmosphere packaging.

Although it has been shown that spoilage can be delayed by removing oxygen (Stammen and others 1990; Penney and others 1994; Hong and others 1996); doing so introduces the danger that apparently unspoiled fish may contain *C. botulinum* toxin. For this reason, the FDA has instituted strict guidelines for the use of ROP for seafood. It is likely that this issue is also central to FDA's reluctance to approve irradiation as a means to extend the shelf life of fresh seafood (Federal Register 1990).

Clostridium botulinum forms toxin more rapidly at higher temperatures than at lower temperatures (Table 1). The minimum temperature for growth of *C. botulinum* type E and nonproteolytic type B and F is believed to be around 3.3 °C (38 °F). As shelf life of refrigerated foods increases, more time is available for *C. botulinum* growth and toxin formation. As storage temperatures increase, time required for toxin formation decreases. The Food and Drug Administration encourages industry to expect that proper refrigeration temperatures will not be maintained during storage, distribution, display or consumer handling of refrigerated foods. Surveys of retail display cases indicate that temperatures of 7 to 10 °C (45 to 50 °F) are not uncommon (FDA 2001). Surveys of home refrigerators indicate that temperatures can exceed 10 °C (50 °F) (FDA 2001).

To assist in identifying potential hazards in food processing and distribution process, FDA offers three factors that are conducive to toxin formation, two of which are related to the packaging (FDA 1998).

(1) Vacuum packaging or modified atmosphere packaging. Because most of these packaging methods exclude or reduce the amount of oxygen in the package, conditions may be favorable for *C. botulinum*

growth and toxin formation; (2) packaging in hermetically sealed containers (such as double seamed cans, glass jars with sealed lids, heat sealed plastic containers) or packing in oil. These and similar processing/packaging techniques prevent the entry of oxygen into the container. Any oxygen present at the time of packaging may be rapidly depleted by the activity of spoilage bacteria, resulting in a reduced-oxygen environment that is favorable for *C. botulinum* growth and toxin formation.

Evidence of the danger of toxigenesis prior to observable spoilage at mildly abusive temperatures has been demonstrated (Post and others 1985). Table 1 summarizes data reported by Reddy and others (1996, 1997a, 1997b). These studies demonstrate the potential of MAP to extend acceptable shelf life, and the remote possibility of toxigenesis prior or coincident to obvious spoilage at mildly abusive temperatures.

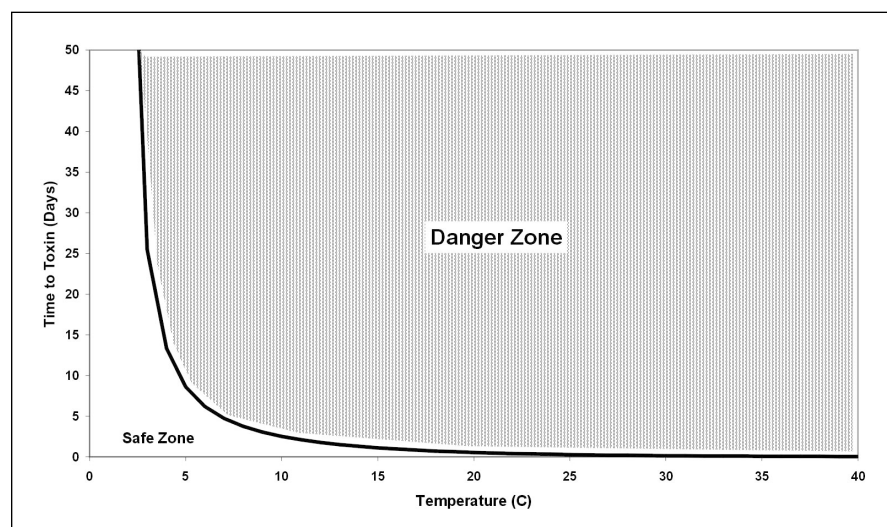
The Skinner and Larkin curve

Recently, Skinner and Larkin (1998) proposed an empirical relationship that provides a conservative prediction for the time required to observe *C. botulinum* toxin as a function of temperature. The Skinner and Larkin relationship is a simplified and more conservative version of an expression proposed by Baker and Gerigeoris (1990). The Skinner and Larkin relationship follows:

$$\text{Log}(L) = 0.65 - 0.0525T + 2.74/T \quad (1)$$

where L is the "lag time" or "time-to-toxigenesis" in d, and T is the temperature in degrees Celsius. Figure 1 shows a plot of the Skinner and Larkin curve.

The Skinner and Larkin curve represents an empirical border around virtually all known conditions where growth of *C. botulinum* have been shown to occur. Therefore, the 2 regions shown in Figure 1 represent

**Figure 1—The Skinner and Larkin (1998) relationship (Eq. 1).**

TTI performance specification...

the best understanding of conditions where *C. botulinum* can and cannot grow.

Role of time-temperature integrators

It is well known that properties of natural and synthetic materials change over time. In the case of foods, particularly refrigerated fresh foods, such changes are generally undesirable, and reflect deterioration of food quality and/or safety. It is also generally recognized that the rate at which such changes occur vary with temperature. For the case of toxin liberation by *C. botulinum*, Eq. 1 describes this temperature sensitivity.

Germination of bacterial spores and the growth of the bacteria are highly complex processes that depend on many factors (Sarathehandren and others 1977). Due to the conservative nature of the Skinner and Larkin relationship, such complex issues may be ignored in order to focus on one critical parameter, namely temperature.

Temperature is often cited as the most important factor affecting food safety and quality (Shimoni and others 2001). Since changes in foods often involve highly complex and poorly understood mechanisms, TTIs are designed using fairly simple physical and/or chemical systems that have well-understood temperature sensitivity characteristics. Therefore, the purpose of a TTI system is to relate a readily observable change in the TTI to changes that are not as readily determined in foods. Specifically, TTIs designed to ensure safety of fresh ROP seafood should allow an observer to relate

Table 2—Comparison of Vitsab M2-10 expiration to Skinner and Larkin relationship (Eq. 1). All values in days.

	Temperature (°C)										
	0	1	2	3	4	5	6	7	8	9	10
M2-10	14.0	12.0	10.0	8.4	7.0	6.0	5.0	4.0	3.5	2.5	2.0
Skinner and Larkin (Eq. 1)	2175.2	82.2	25.5	13.3	8.6	6.2	4.7	3.7	3.0	2.5	

TTI readings to the Skinner and Larkin (1998) relationship (Eq. 1).

Time-temperature integrator performance specification

Commercial TTI vendors often provide “endpoint” data to describe TTI performance. For example, Cox Technologies (Belmont, N.C., U.S.A.) recommends its Vitsab M2-10 for seafood. The designation “M2-10” suggests that the TTI should expire in 10 d at 2 °C. Additional time-temperature performance combinations for the M2-10, as provided by the company, compared with Eq. 1 are shown in Table 2.

Interestingly, information about the path between endpoints is not typically provided. In other words, the current approach to TTI design focuses solely on matching temperature sensitivity of the TTI to the underlying process. Although this approach may be theoretically sound, it may lead to technically correct, yet poorly behaved TTIs that are difficult to interpret subjectively.

Theory

THE RATE AT WHICH TTI READINGS CHANGE with time can often be modeled with

well known kinetic expressions. For instance, the rate of change of and observable TTI parameter, A, can be said to follow the general form:

$$\pm \frac{dA}{dt} = kA^n \quad (2)$$

where k is often referred to as the reaction rate constant, and n is the reaction order. In highly complex systems such as foods, global changes often follow pseudo-zero (n = 0) or pseudo-first order (n = 1) kinetics.

First-order kinetic behavior is often observed in nature. Well-known examples are radioactive decay, and exponential-phase bacterial growth. In such cases it is easy to see that the rate of change of A at any given time is proportional to the magnitude of A at that time:

$$\pm \frac{dA}{dt} = kA \quad (3)$$

Conversely, zero-order behavior is not observed in nature as often. However pseudo-zero order behavior is observed in systems where a potentially limiting component is available in sufficiently excessive amounts that the limitation becomes insignificant. Such behavior is observed in catalyzed reactions in which catalyst concentration is abundant. For the zero-order case, the rate of change is constant:

$$\pm \frac{dA}{dt} = k \quad (4)$$

Solving Eq. 3 and 4 provides relationships that can be used to describe the behavior of zero and first order TTIs between the specified times.

$$n = 0 \quad n = 1$$

$$\pm \frac{dA}{dt} = k \quad \frac{dA}{dt} = \pm kA \quad (5a_0, 5a_1)$$

$$\pm \int_{A_0}^A dA = k \int_0^t dt \quad \int_{A_0}^A \frac{1}{A} dA = k \int_0^t \pm dt \quad (5b_0, 5b_1)$$

$$A = A_0 \pm kt \quad A = A_0 e^{\pm kt} \quad (5c_0, 5c_1)$$

Equations 5c₀ and 5c₁ represent the response paths that zero and first order TTIs would provide. If it is assumed that the response of the Vitsab® M2-10 follows zero

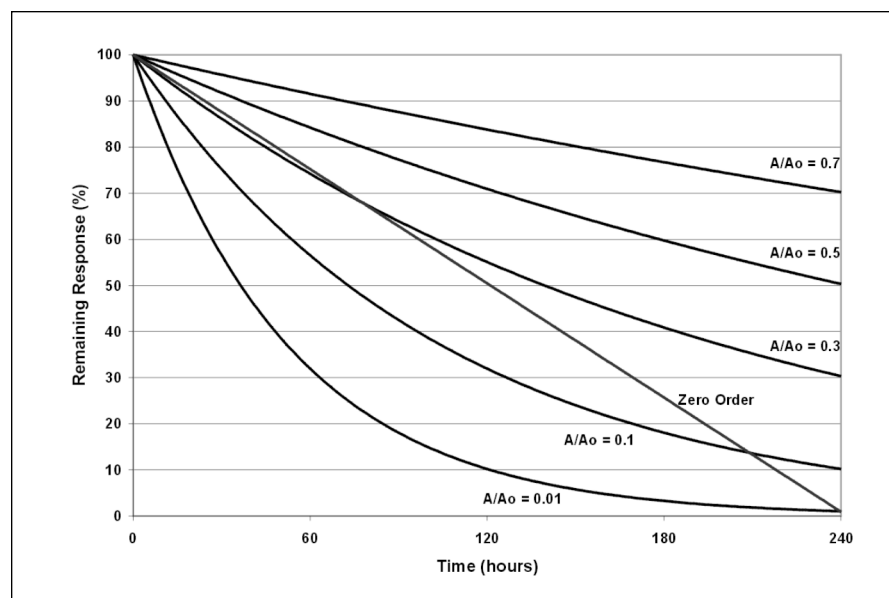


Figure 2—Possible response paths of the Vitsab® M2-10 TTI under constant temperature storage at 2°C, assuming zero and first order kinetics

TTI performance specification...

order kinetics, then the reaction rate at 2 °C would be:

$$k = \frac{A_0 - A}{t} = \frac{100\% - 0\%}{10 \text{ days}} = 10\% \text{ per d} \quad (6)$$

where 100% and 0% refer to the amount of observable response remaining at the beginning and end of the 10 d, respectively. In other words, when the M2-10 is stored at 2 °C, an observer could expect to see a similar amount of response during each of the 10 d until expiration. In this case that amount would be 10% of the total response per day.

If the M2-10 follows first-order kinetics, then we would need to know more about the state of the TTI when it expires in order to be able to determine the reaction rate constant, k , and to predict the path of the response. This is due to the fact that first-order behavior asymptotically approaches the expiration point, and never actually achieves complete expiration. For this reason, the end of the TTIs response must be defined as a fraction of the TTIs total response, A/A_0 . With this additional information, the reaction rate constant may be calculated as follows:

$$k = \frac{\ln(A/A_0)}{t} \quad (7)$$

Figure 2 shows expected response paths for the Vitsab® M2-10 stored at 2 °C, assuming both zero and 1st-order kinetics. For the first order case, several possible endpoint specifications are shown.

Note that all of the curves in Figure 2 satisfy the M2-10 specification, in that they all reach the specified endpoint in 10 d (240 h) at 2 °C. Although each of these paths would provide a conservative response relative to the Skinner and Larkin formula for *C. botulinum*, the differences in the paths raise some practical concerns with regard to actual use. To illustrate, consider the first order TTI for which the end of response is $A/A_0 = 0.01$. Under constant temperature storage at 2 °C an observer would note a fairly rapid response during the first two to three d, but would then see very small changes until expiration. Conversely, for the first order response when the endpoint $A/A_0 = 0.7$, the rate of change would appear to be fairly slow, but consistent. For the zero-order case, the response would appear constant throughout, as expected.

Upon comparing the zero-order and first-order $A/A_0 = 0.7$ response curves it is clear that while both offer consistent changes over time, the overall response of a corresponding first-order TTI would be much more subtle than the zero-order TTI. There-

fore, given a choice, the zero-order behavior should be preferable.

Temperature sensitivity:

As shown, an appropriate assumption of reaction order, n , allows determination of the associated reaction rate constant, k , from endpoint specifications. The Arrhenius relationship (Eq. 8) often describes how k varies with temperature:

$$k = k_0 e^{\left(\frac{-E_a}{RT}\right)} \quad (8)$$

where E_a is the activation energy; R is the ideal gas law constant; T is absolute temperature; k_0 is a constant. Generally, reaction rate constants increase with temperature. The sensitivity of the rate constant to temperature is governed by the magnitude of E_a . For two different reactions, or in this case TTIs, with different E_a values, Eq. 8 dictates that the reaction rate constant with the greater activation energy will change by a greater amount, for a given change in temperature.

Arrhenius parameters, k_0 and E_a , are typically determined from a plot of $\ln(k)$ versus inverse absolute temperature. Equation 9 shows that when such a plot produces a line, the slope is equal to $-E_a/R$ and the intercept is $\ln(k_0)$.

$$\ln(k) = \ln(k_0) - \frac{E_a}{R} \left(\frac{1}{T_{\text{absolute}}} \right) \quad (9)$$

Since it is likely that most commercial TTIs demonstrate Arrhenius temperature sensitivity, it may be useful to transform the empirical Skinner and Larkin (1998) formula (Eq. 1) into a corresponding Arrhenius relationship, so that appropriate comparisons can be made. As previously noted, the reaction order, n , must be assumed in order to convert Skinner and Larkin (1998) lag-times into reaction rate constants.

Physically, the end of the Skinner and Larkin lag-time, L , corresponds to germination of *C. botulinum* spores, growth of bacteria and liberation of toxin. During the lag-time, there are no convenient measurements that can be made to estimate the amount of lag-time already consumed or remaining. The critical and practical aspect is that there must be an absolute understanding that once the safe lag-time is depleted, the ROP food is unconditionally surrendered. In other words, the endpoint is clear, definite, and absolute; an inherent characteristic of zero order kinetics. Moreover, the endpoint is not an arbitrary point on an asymptotic curve; an inherent charac-

teristic of first order kinetics. Therefore, it should be sufficient and conservative to assume that consumption of lag-time follows the most direct path, which is defined by zero-order kinetics. However, this does not mean that the approach described here is restricted to TTIs with zero-order kinetic responses. The approach can be applied equally well to TTIs with first-order, or other types of kinetic responses. However, given the physical nature of the consumption of lag-time, it is suggested that TTIs offering zero-order kinetics are preferable. Therefore, development of the approach below focuses on the case of zero-order kinetics.

Using the zero-order kinetic assumption, Skinner and Larkin (1998) reaction rate constants may be calculated directly from lag-times calculated in a manner similar to that described by Eq. 6:

$$k(T) = \frac{100}{L(T)} \quad (10)$$

Figure 3 shows the Skinner and Larkin (1998) formula in Arrhenius form, assuming zero order kinetics. This curve shall be referred to as S&L-Arrhenius curve.

Prior to applying Figure 3 in the development of methodology appropriate for TTIs, it is important to be able to interpret Figure 3 properly. Since Figure 3 was created with the assumption of zero-order kinetics, Eq. 5a0 dictates that the reaction rate constant, k , is also equivalent to the rate of loss of lag-time. Therefore, when comparing rates of change of zero-order indicator systems to that of the modified S&L-Arrhenius curve in Figure 3, points above the curve represent TTI changes that are faster than consumption of lag-time, while those below represent TTI changes that are slower than consumption of lag time. Since the objective of any TTI is to provide an accurate, yet conservative indication of the consumption of lag-time (or other corresponding process), it is appropriate for zero-order TTI response curves to approach the S&L-Arrhenius curve from above. In other words, it is desirable to be as close to the S&L-Arrhenius curve as possible, but never below the curve.

Methods

THE MANNER IN WHICH THE S&L-ARRHENIUS curve is used to design and/or specify TTI performance depends on the nature of the TTI response. Ideally, one would prefer TTIs to behave identically to the S&L-Arrhenius curve. However, this may be difficult to achieve for any given indicator system. A viable alternative could include TTIs with zero-order response characteristics, and an Arrhenius curve that is as close to the S&L-Arrhenius curve as possible, but without

TTI performance specification...

crossing below the S&L-Arrhenius curve. Figure 4 presents two such Arrhenius curves (lines A and B) that represent the extremes of all possible curves that satisfy this requirement.

Although zero-order TTIs offering the performance depicted by lines A and B (Figure 4) would provide conservative responses relative to the Skinner and Larkin (1998) formula, it is worth noting the practical implications of these extreme cases. Before doing so, it is worth noting 3 points regarding Arrhenius plots. First, increasing values of $\ln(k)$ represent increasing values of the reac-

tion rate constant, k . Second, for the case of zero-order kinetics, the rate constant is equal to the reaction rate. Third, the abscissa of an Arrhenius plot is "inverse absolute temperature," therefore, temperature decreases toward the right and increases to the left.

Consider a TTI with temperature sensitivity described by curve B (TTI-B) in Figure 4. This TTI closely matches the S&L-Arrhenius curve at low temperatures, but becomes increasingly faster (more conservative) as temperature increases. For the case of ROP seafood, TTI-B would perform well

for product distributed and stored under properly controlled temperatures. However, the TTIs would quickly expire under brief exposure to even moderate temperatures. In most cases of slight to moderate temperature abuse, TTI-B would expire well before the underlying products would actually become unsafe to consume. The result being that even insignificant lapses in temperature control during distribution or storage would likely result in significant waste of otherwise safe product.

Now consider TTI-A in Figure 4. Time-temperature integrator-A becomes increasingly conservative (faster) than consumption of lag time at low temperatures. For the case of ROP seafood, TTI-A will expire well before toxigenesis even when product temperature is perfectly controlled throughout distribution and storage. Time-temperature integrators-A would become increasingly accurate as thermally abusive conditions become more severe.

Figure 5 depicts one seemingly straightforward, yet arbitrary and potentially dangerous attempt to compromise between the extremes (curve C), as well as a safer alternative (curve D).

Curve C (Figure 5), can be created via linear regression of either the entire S&L-Arrhenius curve or some arbitrarily selected sub-region. Since it is the purpose of any regression to find the path of least error through a set of data, it should be expected that the resulting line will pass above and below the selected data points (curve C, Figure 5). Clearly, a zero-order TTI with curve C performance would not be conservative within the temperature range where the curve falls below the S&L-Arrhenius curve.

The dangerous region of curve C can be avoided by applying an additive offset of an amount equal to the greatest difference between curve C and the S&L-Arrhenius curve, resulting in curve D (Figure 5). Curve D represents a safe and conservative performance specification for a zero-order TTI, however, it retains the arbitrary nature of curve C, because it depends on the particular range of data used in the regression.

Figure 6 represents a more specific approach that is conservative, practical and not arbitrary. Curve E (Figure 6) is constructed from two points, one that is relevant to the product, and another from the S&L-Arrhenius curve. The result is a curve that provides (1) an appropriate response when temperature control is excellent, (2) the smallest possible excessive response as temperature increases, while (3) ensuring a safe and conservative response under all temperatures.

The product-related point, P1, is defined

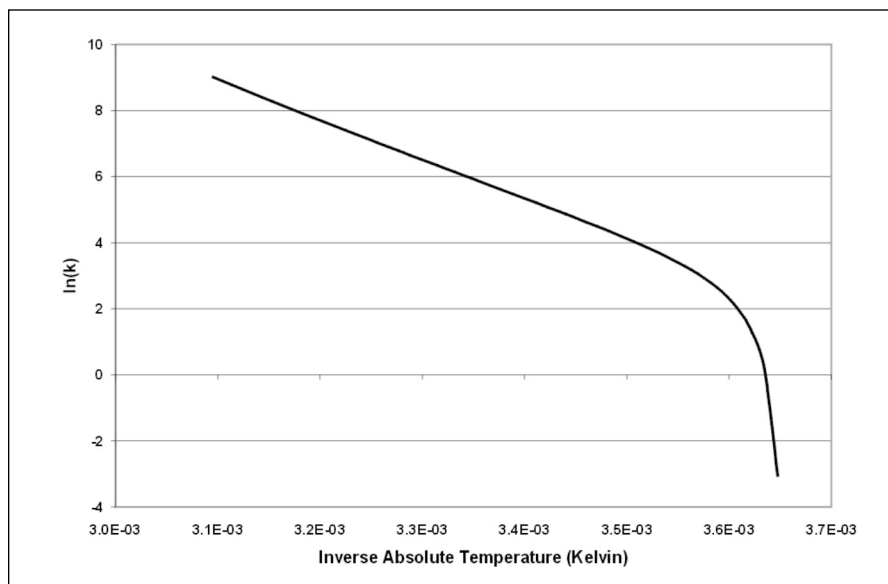


Figure 3—Arrhenius plot of Skinner and Larkin (1998) formula assuming zero-order kinetics

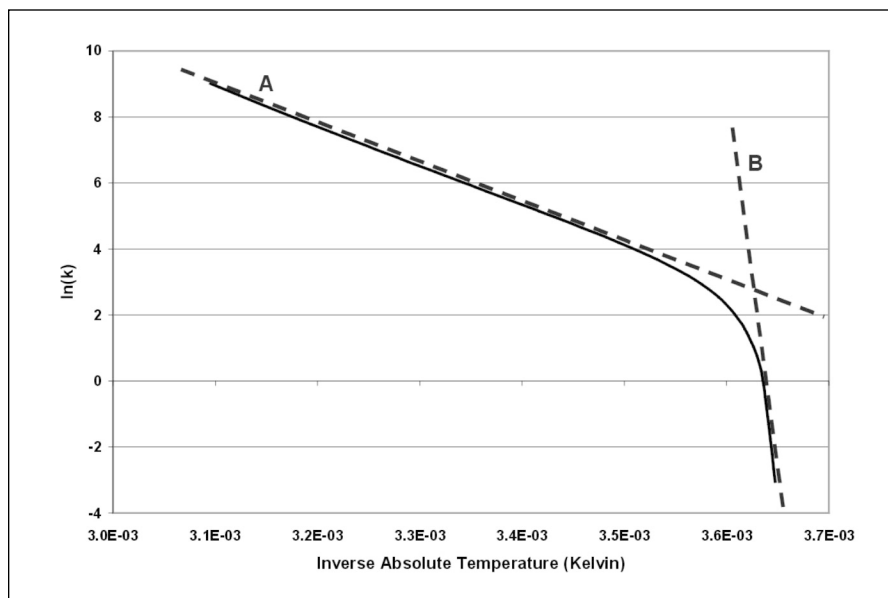


Figure 4—Depiction of extremes for specifying zero-order TTI performance

TTI performance specification...

by the actual shelf life realized under ideal conditions. In other words, P1 represents the maximum achievable shelf-life of the product. For example, if 20 d of shelf-life are achieved at 0 °C, then P1 is defined on the plot as $\{1/273.15, \ln(100/20)\}$. The second point, P2, is defined by the tangent to the S&L-Arrhenius curve that contains P1.

Curve E (Figure 6) offers a practical compromise between realistic shelf life and unnecessary waste of otherwise safe product. A zero-order TTI with curve E performance (TTI-E) provides the appropriate shelf-life at low temperature, accurate safety indica-

tions at moderate temperatures, and conservative indications under increasingly abusive conditions.

Point P2 can be found using many widely available tools such as the Solver feature of Microsoft Excel. However, it is important to note that it is possible to find more than one mathematical solution. Therefore, it is necessary to either discard undesirable solutions, or to constrain the search to a region appropriate to the physical nature of the problem. Figure 7 shows the behavior of the Skinner & Larkin Arrhenius curve over a wider range of temperature values.

For the case of unfrozen ROP seafood, identifying the proper solution or constraining the search for the desired solution is not too difficult, because P1 will likely be defined by temperatures approaching 0 °C ($1/273.15$) from the positive side (fresh fish, by definition, is unfrozen). Therefore, we can expect the desired solution to be at a temperature somewhat above that of P1. For a number of practical situations, constraining the search for P2 to temperatures above 1 °C has proven to be sufficient.

The slope of the tangent to the S&L-Arrhenius curve can be defined by the derivative of this curve. The equation for the slope of the tangent line is

$$f'(x) = -\ln(10) \left[\frac{0.0525}{x^2} + \frac{2.74}{x^2 \left(\frac{1}{x^2} - 273.15 \right)^2} \right] \quad (11)$$

where x is inverse absolute temperature ($1/T_{\text{abs}}$).

Predictive modeling

Figures 8 and 9 show predicted response curves for a zero-order TTI constructed in a manner described by curve E (Figure 6). For this case, point P1 was defined by a product that provides 18 d of shelf life at 1 °C. The tangent to the S&L-Arrhenius curve that contains P1 was found to occur at 8.0 °C. The line passing through these points provides the desired Arrhenius specification for the desired zero-order TTI:

$$\ln(k) = 64.86 - \frac{17311}{T_{\text{absolute}}} \quad (12)$$

Equations 12 and 1 were used to simulate response characteristics under abusive dynamic thermal conditions. In each case, the dynamic thermal condition was generated using the following sine-squared function:

$$T(t) = T_{\text{base}} + A \sin^2 \left(\frac{2\pi t}{P} \right) \quad (13)$$

where T is temperature in °C, T_{base} is a base or minimum temperature of the cycle, A is the amplitude of the cycle (maximum temperature reached in a cycle is $T_{\text{base}} + A$), P is the period of the cycle, and t is time in h. Response values were calculated using a 1-hour time interval. Temperature values equal to 10% above the mean temperature for each interval was used for kinetic response calculations (Welt and others 1997).

Figure 8 depicts a situation in which product is manufactured and packaged under controlled conditions of 1 °C, then transits a poorly controlled distribution chain, but then returns to controlled conditions for

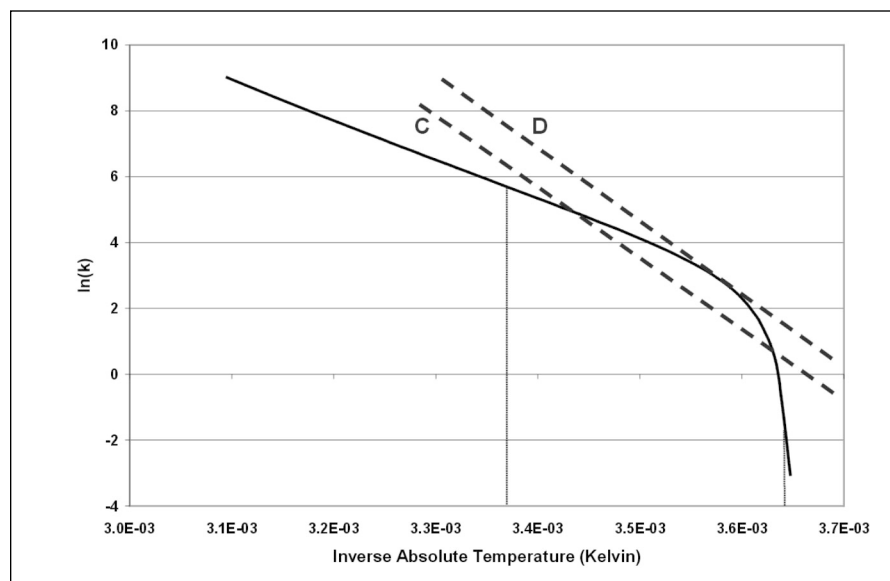


Figure 5—Arbitrary approaches for compromising between extremes shown in Figure 4.

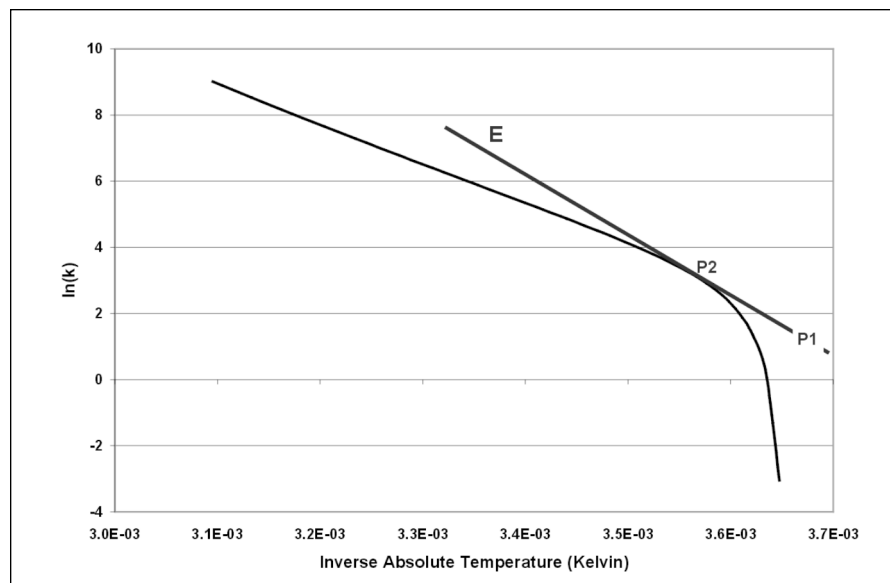


Figure 6—Proposed method for specifying zero-order TTI performance.

TTI performance specification...

storage and/or sale ($T_{\text{base}} = 1^\circ\text{C}$, $A = 8^\circ\text{C}$, $P = 500\text{ h}$).

Figure 9 depicts a daily fluctuation of temperature between 1 and 9°C ($T_{\text{base}} = 1^\circ\text{C}$, $A = 8^\circ\text{C}$, $P = 48\text{ h}$).

As expected, Figures 8 and 9 demonstrate that a zero-order TTI engineered to perform in accordance with Eq. 12 would provide a safe and conservative indication. Under any possible conditions, the TTI could be expected to expire prior to the conservative prediction of toxigenesis provided by the Skinner and Larkin (1998) formula (Eq. 1).

Future research needs

Time-temperature integrator technology offers a promising approach to monitoring product quality and safety. However, a greater understanding of TTI performance attributes is required before confidence in their ability to ensure product safety is achieved. Specifically, procedures need to be established that define how TTIs are to be read, as well as how such readings are to be used. Once such procedures are established, studies need to establish appropriate statistics related to expected performance. These statistics will allow development of limits that will define an appropriate proximity for mean TTI performance to the established border of safety.

Additionally, it is important to understand how external influences might affect TTI performance. Such influences might include (1) handling, storage and shelf-life of the TTIs, (2) location of a TTI on a package, (3) weight of other packages on a TTI, (4) effects of different forms of light in TTIs, and (5) brief exposures of TTIs to environment extremes before and during use.

Conclusion

THE TIME REQUIRED FOR *C. BOTULINUM* spores to germinate, grow and liberate toxin is often reported as a "lag time" or "time to toxigenesis" (Skinner and Larkin 1998). It can be useful to consider this period of time as a consumable resource with specific initial and ending conditions, namely 100% of lag time remaining and 0% of lag time remaining, respectively. The actual path taken between these 2 points is not well understood, but it is not particularly important since the underlying food is safe at all conditions between these 2 points. At the very least, it is conservative to assume that the path taken is a straight line under constant thermal conditions. This consideration provides a useful, but not restrictive performance target for time temperature integrator devices. The additional consideration of the inherent shelf-life of a particular fresh

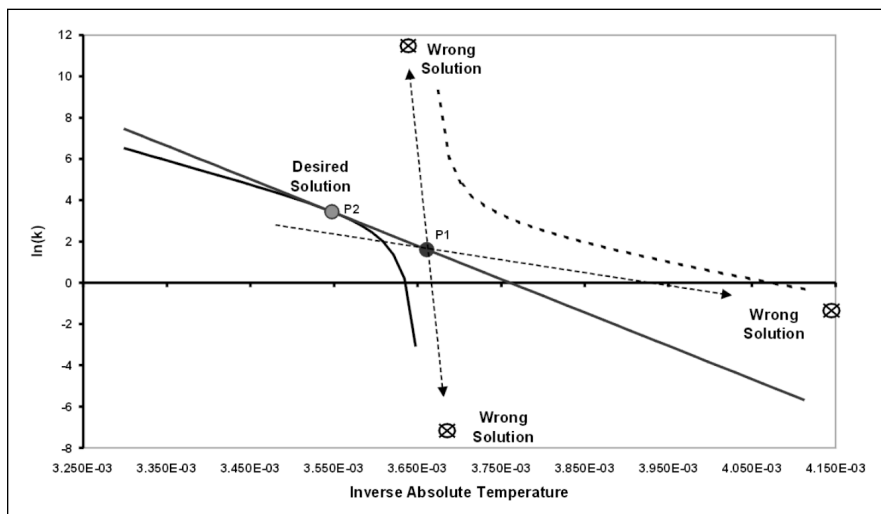


Figure 7—Desired tangent line that contains P1 must either be selected from more than one possible solution, or the search for P2 must be constrained to the region of particular physical interest

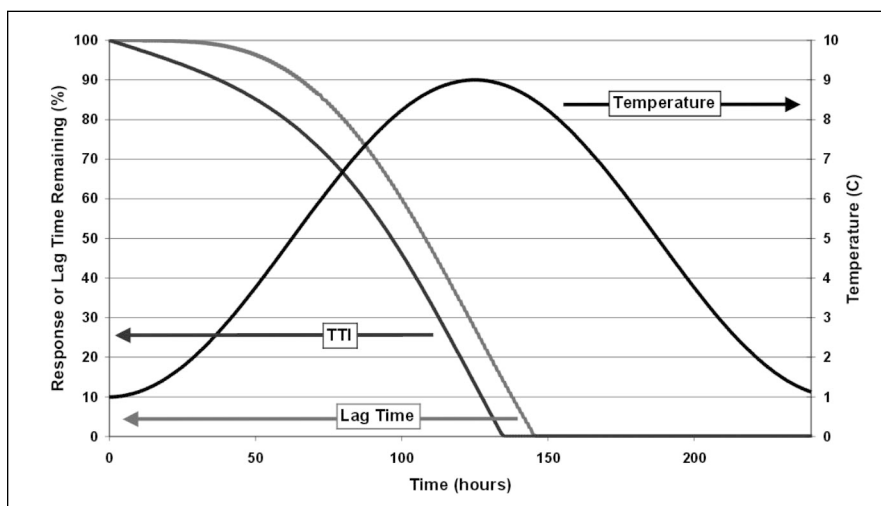


Figure 8—Simulation of TTI performance (P1 specified by 18-d shelf-life at 1°C) versus Skinner & Larkin (1998) lag-time under abusive distribution conditions.

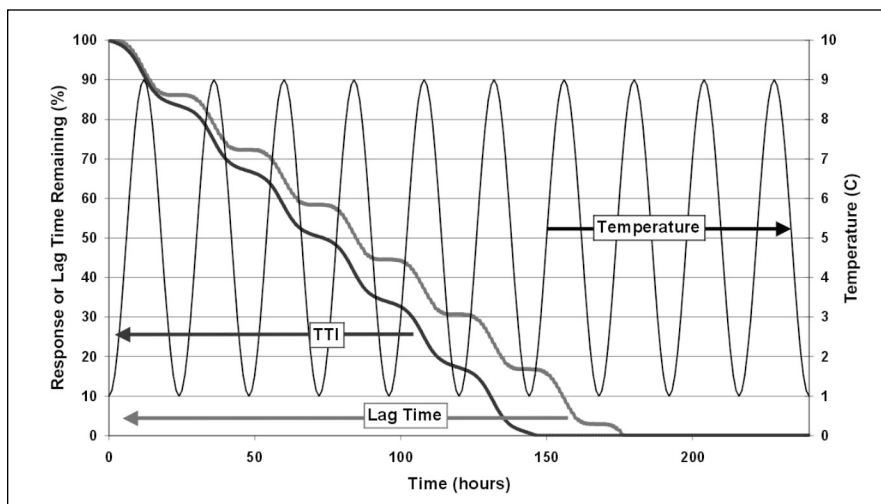


Figure 9—Simulation of TTI performance (P1 specified by 18-d shelf-life at 1°C) versus Skinner & Larkin (1998) lag-time under abusive daily temperature cycles.

food packaged in a reduced-oxygen environment, provides a safe and practical product-specific TTI performance specification.

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