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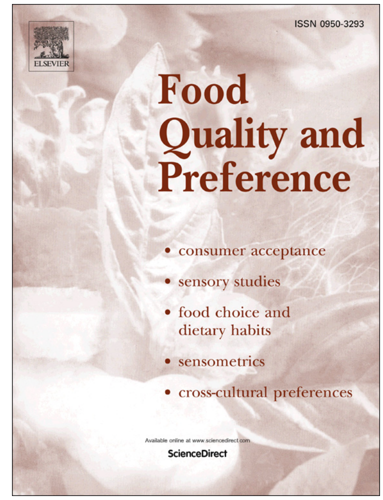

Survival analysis model to estimate sensory shelf life with temperature and illumination as accelerating factors

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Title

Survival analysis model to estimate sensory shelf life with temperature and illumination as accelerating factors

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Abstract

The main objective of this study was to introduce a survival model to contemplate two simultaneous accelerating factors affecting a food product's shelf life: temperature and illumination. A second objective was to consider the case where the same consumer tests different experimental conditions and thus his/her data are not independent. Sample data comprised 108 consumers who evaluated a lemon-flavored juice stored at 24ºC, 37ºC and 45ºC; under conditions of no-illumination and with illumination; with seven different storage times for each of the six experimental conditions. Aiming to estimate the storage time at which a consumer rejects a sample a model including an Arrhenius term for the temperature, a binomial response for illumination (with and without) and the interaction of both was developed. The model also considered that the same consumer tested different experimental conditions.

Keywords: sensory; survival analysis; shelf life; accelerated studies; temperature; illumination.

1 Introduction

In developing new food products companies often want these products on the market in record time, before there is time to estimate their shelf lives. Time to test the products may be reduced to a days or weeks when the expected shelf lives are weeks or months, respectively. Accelerating variables can applied at high levels to then extrapolate estimations to lower levels. For example, temperature has been used to accelerate changes during storage in tomato concentrate (Pedro & Ferreira, 2006) and different levels of temperature and light have been used to estimate the shelf life of olive oil (Manzocco, Panozzo &Calligaris, 2012).

The most widely used accelerating factor is temperature. Variation of rejection behavior in relation to storage time and temperature was modeled by Hough, Garitta, & Gómez (2006). The sensory shelf-life (SSL) of minced meat appearance was taken as a case study. Garitta, Hough, & Sánchez (2004) also used temperature as the accelerating factor in the estimation of dulce de leche's SSL, based on a cut-off point methodology which related consumers' acceptability to trained panel measurements of the critical descriptor.

Accelerating factors other than temperature can be important in certain food products. Humidity can be critical to crisp products such as biscuits and snacks. For example Lee & Resurreccion (2006) presented a study where both temperature and humidity were analyzed as accelerating factors in SSL for roasted peanuts. Oxygen concentration could represent a potentially exploitable accelerating factor in foods susceptible to oxidative deterioration, however little has been published on this topic (Calligaris, Manzocco, Anese, & Nicoli, 2016).

Another accelerating factor important in some food products is light. Manzocco, Kravina, Calligaris, & Nicoli (2008) studied the effect of light and temperature on a solution of saffron, applying four temperatures and four levels of illumination. They measured changes in color using a colorimeter and chemical changes by chromatography. They found that the temperature activation energy was inversely related to illumination intensity. Color changes in brandy over time as a function of temperature and illumination were measured by Espejo & Armada (2014). They considered three storage temperatures and two illumination conditions, with and without. Color changes were measured using a colorimeter. Illumination affected brandy color significantly, with the particularity that color changes were more pronounced at the lowest storage temperature. Manzocco et al. (2012) measured peroxide value changes in vegetable oils during storage time under different temperatures and illumination conditions.

They presented a model which integrated the effect of both accelerating factors. Similar studies to these already mentioned have been applied to honey (Boonchiangma, Chanthai, Srijaranai, & Srijaranai, 2011), jujube fruit (Jiang, Zheng, & Lu, 2014) and paprika (Koncsek, Kruppai, Helyes, Bori, & Daood, 2016). In all these publications the degradation of color or other factors over storage time due to different illumination conditions were measured chemically or physically, but not by sensory analysis.

There have been few publications where sensory evaluation was used to measure the effect of light on shelf life of foods. Jensen, Sørensen, Engelsen, & Bertelsen (2001) measured the effect of light on walnuts stored at 5ºC and 21ºC under an accelerating atmosphere of 50% oxygen. They included flavor descriptors measured by a trained sensory panel but did not estimate shelf life under different conditions, limiting their conclusion that it is best to store walnuts at 5ºC in the dark. Sanz Cervera, Olarte, Echávarri, & Ayala (2007) studied the effect of light and packaging on shelf life of cauliflower stored at 4ºC. They estimated shelf life based on an arbitrary cut-off point on a quality scale used by a trained panel of 7 members who also measured acceptability. A similar panel was used by Zhan, Hub, Li, & Pang (2012) in estimating shelf life of broccoli under different illumination and temperature storage conditions. Ramírez, Hough & Contarini (2001) estimated shelf-life of sunflower oil at different temperatures, with and without illumination. They applied a consumer-based cut-off point for their shelf-life estimation, but with the introduction of survival analysis this cut-off point methodology has now evolved (Garitta, Langohr, Gómez, Hough, & Beeren, 2015).

Survival analysis (Meeker & Escobar 1998; Klein, & Moeschberger, 1997) encompasses a range of statistical methods used in areas such as clinical studies, epidemiology and reliability studies. Hough, Langohr, Gómez, & Curia (2003) introduced survival analysis as a means of estimating SSL based on consumer’s rejection of aged samples. Another application has been to estimate optimum concentrations of a food ingredient, as done by Esmerino, Paixão, Cruz, Garitta, Hough & Bolini (2015) to estimate the optimum sucrose concentration in probiotic petit suisse cheese. As mentioned above, Hough et al. (2006) developed an accelerated sensory SSL model based on survival analysis statistics where the accelerating factor was storage temperature. Meeker & Escobar (1998) present models which allow for more than one accelerating factor, for example a life test of glass capacitors at higher than usual levels of temperature and voltage; or humidity and temperature in materials suffering corrosion. These survival models with more than one accelerating factor have not been applied to food products.

When performing a shelf-life consumer test involving the acceptance or rejection of samples of a single product stored for different times, the first step in survival analysis of the data is establishing each consumer's censorship (Hough, 2010). For example, if 100 participants participated in the test, there could be 10 whose data are not considered because they rejected the fresh sample, and the remaining 90 will each have either left, right or interval censored data. The 90 rows, each corresponding to a single consumer, will be taken up by the survival procedures of a specific program such as R to estimate the product's shelf life. However, there are projects that involve different formulations of the product or different storage conditions of the same product. In these cases it can be convenient to have the same consumer test several sets of samples. For example, Hough et al. (2006) performed an accelerated study on minced meat stored at 2ºC, 9ºC and 19ºC. For each temperature there were 7 samples corresponding to different storage times; thus a total of 3 temperatures X 7 storage times= 21 samples. As the interest was on the appearance only, it was convenient to have each consumer evaluate all 21 samples in a single session. That is, the same consumer tested the three temperature conditions. This meant there was a blocking effect of the consumer in the design which in that paper was not considered. One way to handle this blocking or non-independence effect is to use the working independence approach to estimate the model parameters and the Sandwich estimator for the computation of the corresponding standard errors. This approach, is known as a generalized estimation equation approach in generalized linear models (Hardin, & Hilbe, 2013). It is preferable to a mixed effects model when the interest is to study the average rather than the subject-specific effects of the covariates (Gardiner, Luo, & Roman, 2009).

Objectives of this study were: (a) to introduce a survival model to contemplate two simultaneous accelerating factors affecting a food product's shelf life, and (b) consider the case where the same consumer tests different experimental conditions and thus his/her data are not independent.

2 Materials and Methods

2.1 Samples and storage conditions

The study was performed on a lemon-flavored drink manufactured by a leading beverage company in Argentina. This product was chosen due to ease of storage and sample presentation, and also due to the fact that the manufacturer contributed financially to the project. The drink was packaged in 500 ml PET bottles and was stored at 24ºC, 37ºC and

45ºC for the storage times shown in Table 1. At each of these temperatures samples were stored with no illumination and illuminated 12 hours a day with a day-type fluorescent lamp to simulate conditions of bottles on supermarket shelves. To estimate the luxes received by bottles on supermarket shelves, lux measurements were carried out with a luximeter (TES-1330A Digital Light Meter, TES Electrical Electronic Corp., Neihu Taipei, Taiwan) in three supermarkets in the town of 9 de Julio, Buenos Aires, Argentina. The three supermarkets were from a nationwide chain, a regional chain and a local store. Three shelves were considered: low, middle and high; taking the measurement adjacent to the aisle.
Average measurements over the three supermarkets were: 60, 125 and 240 luxes for the lower, middle and upper shelves, respectively. In view of these measurements, we adjusted the illumination of our storage chambers to be between 160 and 190 luxes; the range was because illumination was not perfectly uniform. Bottles were rotated every 10 days.
There were a total of six experimental conditions corresponding to the combination of temperature (24ºC, 37ºC and 45ºC) and illumination (No and With). As shown in Table 1, for each of these conditions there were 7 storage times. The design corresponded to reverse storage to ensure all storage times for a given condition could be evaluated in a single session (Hough, 2010). All samples came from a single batch which was stored at 2ºC in the dark to ensure no changes till transferred to one of the above storage conditions.

2.2 Consumer study

Hough, Calle, Serrat, & Curia (2007) presented graphs which allowed estimation of the number of consumers necessary for shelf-life estimations based on survival analysis statistics. They presented the case of choosing an average σ, an alpha value (Type I error) of 5%, a beta value (Type II error) of 20%, the shelf life to be in the middle of the studied time range, and a difference between the true shelf life and the estimated shelf life of 0.5 on a 0–6 time scale; with these parameters the estimated number of consumers would be 120.
Consumers of fruit-flavored drinks who did not reject lemon flavor were recruited for the study. Ages were between 18 and 60 years, and there were approximately half female. 108 of the 120 initial consumers turned up to complete the six sessions. The order in which the experimental conditions were assigned to sessions was randomized. In each session they evaluated 6 samples with different storage times (all times except 0) corresponding to one of the temperature and illumination conditions. Presentation order of the 6 samples were balanced over consumers. As the sample corresponding to time= 0 was the same for all conditions, it was included in only one of the sessions. 50 ml of each sample were served in

70 ml plastic glasses coded with three-digit numbers. For each of the samples they had to answer if they would normally consume the product, yes or no.

Twenty-five of the 108 consumers rejected the fresh sample, thus 83 consumers were retained for shelf-life estimations. This number is below the 120 recommended consumers indicated above (Hough et al., 2007); however, as the objectives of the present work were related to model development and not a representative estimate of the lemon drink's shelf life, they were considered sufficient. The raw accept/reject data were processed to obtain the censored data for each consumer and storage condition. Table 2 shows the censored data corresponding to one of the consumers.

2.3 Model considering temperature and light as accelerating factors.

Meeker & Escobar (1998, section 18.5) proposed accelerating models with more than one accelerating variable. Supposing that the storage time at which a consumer rejects a sample ($Time_R$) has a log-location-scale distribution, then:

$$Y = \ln(Time_R) = \beta_0 + \frac{E_a}{R} \times \frac{1}{Temp} + \beta_1 \cdot I + \beta_2 \cdot I \cdot \frac{1}{Temp} + \sigma W \quad (1)$$

Where: $Time_R$= storage time at which a consumer rejects a sample;

β_0, β_1 and β_2 are regression coefficients

E_a= activation energy, cal/mol

R= gas-law constant= 1.98 cal/(mol.ºK)

Temp= storage temperature, ºK

I= illumination condition, 0 for no-illumination and 1 for with-illumination.

σ is the scale parameter and W is the error distribution.

In shelf-life experiments consumers receive samples with different storage times. Consider $Time_R$ as the storage time corresponding to a consumer rejecting a sample. The rejection function F(time) is the probability of a consumer rejecting the sample before 'time', that is F(time)) = P($Time_R \leq$ time). Klein & Moeschberger (1997) propose different distributions for $Time_R$, for example, the log-normal or the Weibull distribution. If the Weibull distribution is chosen, Equation (1) can be combined with the Weibull model and the rejection probability is expressed as:

$$F(time) = 1 - \exp\left[-\exp\left(\frac{\ln(time) - \mu}{\sigma}\right)\right] = 1 - \exp\left[-\exp\left(\frac{\ln(time) - (\beta_0 + \frac{E_a}{R} \times \frac{1}{Temp} + \beta_1 \cdot I + \beta_2 \cdot I \cdot \frac{1}{Temp})}{\sigma}\right)\right] \quad (2),$$

The likelihood function, which is generally used to estimate the rejection function, is the joint probability of the given observations of the n consumers (Klein & Moeschberger, 1997):

$$L = \prod_{i \varepsilon R} (1 - F(r_i)) \prod_{i \varepsilon L} F(l_i)) \prod_{i \varepsilon I} (F(r_i) - F(l_i)) \qquad (3)$$

where R is the set of right-censored observations, L the set of left-censored observations, and I is the set of interval-censored observations.

Equation (2) is a log-linear model, with F(time) being the dependent-y variable; and time and I the independent-x variables. The parameters to be estimated are: β_0, β_1,β_2, Ea and σ; they are obtained by maximizing the likelihood function expressed in Equation (3) (Hough et al., 2003). This is done by the use of statistical software applied to given experimental data. In the present work the R Statistical Package (http://www.r-project.org/, accessed May 3 2017) was used.

Within R, the `survreg` function of the survival package was used (Therneau, 2017) taking into account that each one of the storage conditions were evaluated by the same group of consumers by applying the working independent approach as mentioned in the Introduction (Hardin & Hilbe, 2013). This is accomplished with the `cluster` function as shown in the following R code snippet (refer to Table 2 for abbreviations):

```
> library(survival)
# Fit of a Weibull regression model
> survreg(Surv(lti, uti, censorship, type = "interval") ~ (1/(Temperature + 273) *
Illumination + cluster(Consumer), data = drink)
```

To test whether there is a Temperature-Illumination interaction, that is, to test the null hypothesis H_0: $\beta_2 = 0$, the likelihood ratio test can be used. Its test statistic is the difference of the deviances of the model under the null hypothesis and of the full model, respectively, which follows a chi-squared distribution with one degree of freedom under the null hypothesis.   The same test, or, alternatively, the Wald test, can be used to test whether the storage time until rejection depends on temperature and/or illumination. Three different parametric models were considered: the Weibull, the log-logistic, and the lognormal Statistical significance was set at 0.05. Once the model with the significant terms was chosen, the distribution with the lowest absolute loglikehood value was chosen (Hough, 2010).

3 Results

The likelihood ratio tests showed that the interaction term of Equation (1), that is Illumination.1/Temp, was not statistically significant. The other two terms, temperature expressed as 1/Temp and illumination (no-illumination and with-illumination), were both statistically significant. This conclusion was the same applying any of the three distributions: Weibull, log-normal or normal. Comparing the loglikelihoods of the Weibull, log-normal and normal distributions, each with temperature + illumination as covariates, showed that the Weibull had the lowest absolute value and was thus the chosen distribution.

The estimation of the parameters corresponding to the Model in Equation (1) excluding the interaction are shown in Table 3. The positive sign of $\widehat{Ea}$ and the negative sign of $\hat{\beta}_1$ indicate, respectively, that, on average, storage times until rejection decrease as the storage temperature increases and are shorter in the case of illumination.

With these parameters, Equation (2) can be used to estimate percent rejection as a function of storage time given a chosen temperature and illumination conditions. For example, if we consider a storage temperature of 24ºC, rejection probabilities under conditions of no-illumination and with- illumination can be expressed as:

- No-illumination:

$$F(time) = 1 - \exp\left[-\exp\left(\frac{\ln(time) - \left(-23.66 + \frac{8881}{(273+24)} - 0.15 \cdot 0\right)}{0.596}\right)\right] = 1 - \exp\left[-\exp\left(\frac{\ln(time) - 6.242)}{0.596}\right)\right]$$

- With-illumination:

$$F(time) = 1 - \exp\left[-\exp\left(\frac{\ln(time) - \left(-23.66 + \frac{8881}{(273+24)} - 0.15 \cdot 1\right)}{0.596}\right)\right] = 1 - \exp\left[-\exp\left(\frac{\ln(time) - 6.092)}{0.596}\right)\right]$$

These expressions are plotted in Figure 1. As expected, for the same storage time, % rejection under the condition with-illumination is predicted to be higher than under no-illumination.

In predicting SSL, an acceptable rejection probability has to be adopted to estimate the corresponding storage time. A generally accepted value (Hough, 2010) is the 50% rejection probability at the end of the product's shelf life. For this purpose, R function `predict` can be applied upon the `survreg` function as in the following example:.

```
# Model-based prediction of the 10%, 25%, and 50% quantile
> predict(survreg(Surv(lti, uti, censorship, type = "interval") ~ (1/(Temperature +
  273) * Illumination + cluster(Consumer), data = drink), newdata, type =
  "quantile", p = c(.1, .25, .5), se.fit = TRUE)
```

The above mentioned R function estimates the 10, 25, and 50% quantiles for temperature and illumination conditions specified in `newdata`. Table 4 presents the estimates corresponding to the experimental conditions used in the present study for 25% and 50% quantiles. Storage temperature had an important effect on SSL.

In practical situations it is of interest to know how the deterioration rate changes from one temperature-illumination condition to another. This can be expressed as the accelerating factor (AF, Meeker & Escobar, 1998, section 18.5) defined as:

$$AF = \frac{\text{reaction rate at accelerated temperature and illumination condition } I_a}{\text{reaction rate at usage temperature and illumination condition } I_u}$$

Referring to Equation (1), AF can be expressed as:

$$AF = \frac{\exp\left[\frac{Ea}{R} \times \frac{1}{Temp_{accel}} + \beta_1 \cdot I_{accel} + \beta_2 \cdot I_{accel} \cdot \frac{1}{Temp_{accel}}\right]}{\exp\left[\frac{Ea}{R} \times \frac{1}{Temp_{usage}} + \beta_1 \cdot I_{usage} + \beta_2 \cdot I_{usage} \cdot \frac{1}{Temp_{usage}}\right]} \qquad (4)$$

When the illumination conditions in the accelerated and usage conditions are the same, and the temperature difference $Temp_{accel} - Temp_{usage} = 10$ºC, Equation (4) is reduced to the classical Q_{10} (Labuza, 1982) which expresses the change in reaction rate with a 10ºC temperature difference and is often preferred to Ea as it is ea is easier to interpret:

$$Q_{10} = e^{\frac{E_a}{R} \times \frac{10}{T(T+10)}} \qquad (5)$$

By using Equation (5, Q_{10} has different values according to what temperature is chosen. Within the experimental range of this experiment, Q_{10} values were estimated to be 2.59 and 2.44, at 30ºC and 40ºC, respectively. Thus for every 10ºC reduction in storage time, SSL is more than doubled.

Figure 1 and Table 4 both show that shelf life was reduced when the product was exposed to illumination. This is in line with other products mentioned in the Introduction studied with and without illumination during storage, such as saffron, jujube fruit, paprika, walnuts and vegetable oil. The lemon-flavored juice used in the present study had lemon juice and lemon flavoring declared as part of its ingredients and it is well documented that the sensory properties of lemon flavored drinks change over storage time. Some authors attribute these changes the transformation of D-limonene to carvona and carveol (Braddock, 1986). The limonene easily degrades by oxidation reactions at acidic environment contributing to an undesirable off-flavor (Carmo, Pais, Simplicio, Mateus, & Duarte, 2017). The cause of this change is oxidation originated by UV photolisis which is accelerated by temperature (Nguyen, Campi, Roy Jackson, & Patti, 2009). Other authors attribute changes mainly to citral's transformation to p-cymene, p-cresol and dimethyl-styrene (Sawamura, 2004). Both limonene and cytral changes lead to sensory deterioration of the product as observed in the present study.

Table 4 shows that in absolute value standard errors of SSL estimations were higher for lower storage temperatures. However, if the standard error is adjusted by dividing its value by the estimation, analogous to a coefficient of variation, it can then be seen that estimation errors were similar for the different storage temperatures as shown in Table 5 both for 25% and 50% rejection probabilities.

The estimates were obtained based on a sample of 108 consumers, of which the data of 83 were considered (Section 2.2). This number was below the recommended value of 120 (Hough et al., 2007). In this last paper, the estimation of 120 was based on finding a difference between the true shelf life and the estimated shelf life of 0.5 on a 0–6 time scale. The coefficient of variation will depend on the estimated shelf-life value. For 3 and 6 on the 0-6 scale the coefficients are 16.7% and 8.3%, respectively. Values in Table 5 are within this range. It should be noted that in the present study each consumer evaluated six experimental conditions and were each considered as a block. This experimental design was not considered in Hough et al's (2007) paper; thus estimations are not directly comparable.

4 Conclusions

In this study, as in previous papers (Hough et al., 2003; Hough et al., 2006), shelf-life estimations are based on the probability of consumer rejection after a certain storage time. In the present paper a model based on two accelerating factors- temperature and illumination- affecting a food product was introduced. This type of model with two accelerating factors has not been considered in previous publications. The model included an Arrhenius term for the temperature, a binomial response for illumination (with and without) and the interaction of both. The response was the censored data obtained from accept or reject responses obtained from consumers who evaluated samples with different storage times. A limitation of the present study was the binomial response for illumination, a further study should include experiments with variations in light intensity to thus include this variable as continuous in the model.

An important addition to the model was the consideration that the same consumer tested different experimental conditions. This blocking or non-independence effect has not been considered in previous publications.

Classical likelihood ratio test was applied to analyze the significance of each one of the model's terms; for the particular lemon-flavored juice data used in this study the temperature and illumination main effects were significant, but not their interaction. Activation energy corresponding to consumers' rejection of the product stored at different temperatures was calculated, accompanied by the easier to interpret value of Q_{10}. For the particular product used to test the model, illumination accelerated deterioration, resulting in a higher rejection probability in relation to no-illumination conditions. Estimated SSL values can be obtained from the model with their corresponding standard errors.

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Figure captions:

Figure 1. Predicted percent rejection in relation to storage time for a lemon-flavored juice stored at 24ºC under conditions of no-illumination and with-illumination.

Table 1. Storage times of the lemon-flavored drink at different temperatures.

Days at 24ºC	Days at 37ºC	Days at 45ºC
0	0	0
90	35	14
150	59	28
210	80	42
210	94	49
270	108	55
300	119	60

Table 2. Censored data corresponding to one of the consumers for different storage temperatures and illumination.

Consumer	Lower time interval (lti-days)	Upper time interval (uti-days)	Type of censorship	Temperature (ºC)	ILLUMINATION
1	300	300	right	24	No
1	150	270	interval	24	With
1	35	119	interval	37	No
1	119	119	right	37	With
1	49	55	interval	45	No
1	42	49	interval	45	With

Table 3. Parameter estimations corresponding to the Weibull distribution with the inclusion of temperature and illumination as covariates (see Equation (2)).

Parameter	Estimation	Standard error
β_0	-23.66	1.412
Ea (cal/mol)	17584	865
β_1	-0.15	0.0705
σ	0.596	0.0543

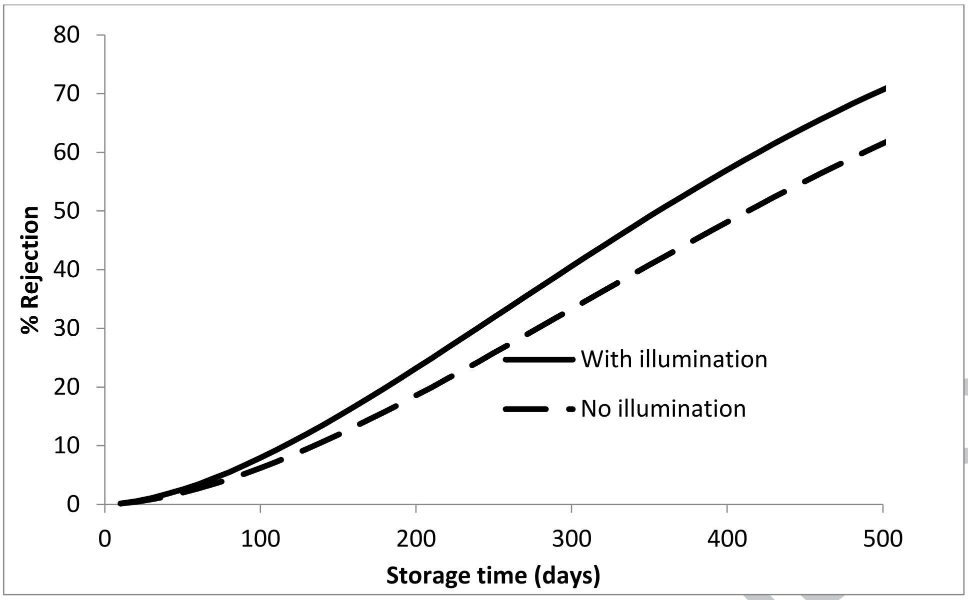
Table 4. Lemon-flavored drink sensory shelf-life estimations (days ± standard error) corresponding to a 50% rejection probability for the experimental storage conditions of temperature and illumination

Temperature (ºC)	No-illumination		With-illumination	
	25%	50%	25%	50%
24	244 ± 21	412 ± 34	210 ± 20	354 ± 29
37	67 ± 5.7	117 ± 7.5	60 ± 5.5	101 ± 6.4
45	34 ± 3.4	57 ± 4.4	29 ± 3.1	49 ± 3.8

Table 5. Coefficients of variation expressed in percentage obtained by dividing standard errors by their corresponding estimation values

Temperature (ºC)	No-illumination		With-illumination	
	25%	50%	25%	50%
24	8.5	8.2	9.4	8.2
37	8.1	6.3	9.1	6.3
45	9.9	7.7	10.7	7.6

Figure 1.


80
70
60
50
40
30
20
10
0
% Rejection
0
100
200
300
400
500
Storage time (days)
With illumination
No illumination

HIGHLIGHTS

We developed a survival model which considered two accelerating factors: temperature and illumination

The blocking effect of the consumer was considered in the survival model

The model was applied to real data from a lemon-flavored drink sensory shelf-life

As expected shelf life was shortened with an increase in temperature and under illumination