CURRENT-STATUS SURVIVAL ANALYSIS METHODOLOGY APPLIED TO ESTIMATING SENSORY SHELF LIFE OF READY-TO-EAT LETTUCE (*LACTUCA SATIVA*)

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ABSTRACT

The objective of the present work was to develop a method for predicting sensory shelf life for situations in which each consumer evaluates only one sample corresponding to one storage time. This type of data is known as current-status data in survival analysis statistics. The methodology was applied to estimate the sensory shelf life of ready-to-eat lettuce (Lactuca sativa *var.* capitata *cv.* "Alpha"). *For each of six storage times, 50–52 consumers answered yes or no to whether they would normally consume the presented sample. The results were satisfactory, showing that the methodology can be applied when necessary. The Weibull model was found adequate to model the data. Estimated shelf lives ± 95% confidence intervals were 11.3 ± 1.2 days and 15.5 ± 0.9 days for a 25% and a 50% consumer rejection probability, respectively.*

PRACTICAL APPLICATIONS

When considering shelf-life evaluations by consumers, the first idea is to have each consumer evaluate six or seven samples with different storage times in a single session. To do this, a reverse storage design is necessary, and in the case of a product such as lettuce, it would lead to different batches being confused with storage times. The methodology proposed in this article avoids this problem by having each consumer evaluate a single sample. Another issue with consumers tasting several samples in a single session is how representative this situation is of real consumption. The present methodology allows for

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a consumer to take home, e.g., a bottle of beer with an established storage time, and later collecting the information as to whether they found the beer acceptable or not. This is a situation much closer to real consumption.

INTRODUCTION

Ready-to-eat (RTE) vegetables are those that have been cut, washed and stored in such a way as to combine convenience with freshness (Cantwell and Suslow 2002). The biggest market of this type of vegetables corresponds to fresh salads, of which lettuce is usually an important ingredient. One of the main objectives in the processing of these vegetables is to ensure the absence of pathogenic bacteria. Once this hurdle has been surpassed, the shelf life of the product will depend mainly on its sensory properties.

Kim *et al.* (2005) used three trained assessors to determine the shelf life of modified-atmosphere-packaged lettuce, arbitrarily fixing a score of 3 (strong) on a 0 (none) to 4 (severe) off-odor scale, as not acceptable. There is no mention in this article of having considered consumers' opinions in adopting this cutoff point. A similar scale and arbitrary cutoff point was used by McKellar *et al.* (2004) in their study on the influence of chlorinated water treatment and packaging on the shelf life of RTE lettuce.

As discussed by Hough *et al.* (2003), food products do not have sensory shelf lives of their own; rather they will depend on the interaction of the food with the consumer. For example, Jacxsens *et al.* (2002) reported a sensory shelf life of 7 days for lettuce stored in an equilibrium modified-atmosphere package at 4C. This value was obtained with 8–10 trained assessors who used a freshness scale going from 1 (excellent fresh) to 10 (extremely deteriorated); the sample was considered unacceptable when a mean score above 5 was reached. This lettuce stored for 7 days will be rejected by one consumer who is very fussy over the freshness of his or her lettuce and accepted by another consumer who does not mind a wilted lettuce leaf. Hough *et al.* (2003) applied survival analysis statistics to determine sensory shelf life based on consumer acceptance or rejection of products with different storage times. Their key concept was to focus the shelf-life hazard on the consumer rejecting the product, rather than on the product deteriorating.

Wei *et al.* (2005) studied acidified warm water treatment and Garcia *et al.* (2003) investigated ozone and chlorine treatment of RTE lettuce. In both cases, panels of 30 or fewer subjects were used to measure sensory acceptability over the shelf-life period. Measuring sensory acceptability with a reduced number of subjects is not recommended (Hough *et al.* 2006c), yet researchers do so because it is difficult to repeatedly assemble a large group of consumers over the period of shelf-life studies.

In previous applications of survival analysis statistics to shelf life of foods, it was possible to present samples with different storage times to consumers in one session (Hough *et al.* 2003; Curia *et al.* 2005; Hough *et al.* 2006a). This implies the applicability of reverse storage designs (Hough *et al.* 2006b). If an RTE lettuce study was to be conducted at a storage temperature of 4C, a first batch of vegetable would be placed at 4C and this would correspond to the longest storage time. A second batch, harvested 3 days later, would be placed at 4C and this would correspond to the second longest storage time. This process would continue until all storage times have been completed. This system has the advantage of being able to measure all samples on the same day, but has the disadvantage of having storage times and batches confused. For lettuce and most other vegetables, this would be the case as batches are variable from one harvesting time to another. The other reverse storage design uses a single batch of product, but as they are removed from storage they are frozen so they can all be evaluated together at a future date. Lettuce and other vegetables suffer considerably if frozen.

The other alternative is to have the same consumers perform repeated tests for each one of the storage times as was carried out for a study on tomato maturity times (Garitta *et al.* 2008). Assembling the same group of consumers on six or seven occasions corresponding to each storage time can be cumbersome, unreliable and costly. These consumers soon realize they are participating in a shelf-life study that introduces an expectation error, whereby they feel they have to start rejecting samples somewhere along the line.

From the above discussion, it can be concluded that a method to predict sensory shelf life, in which each consumer evaluates only one sample corresponding to one storage time, would be of value. The objectives of the present work were to develop such a method and to apply this methodology to estimate the sensory shelf life of RTE lettuce.

MATERIALS AND METHODS

Lettuce Samples and Treatments

The samples of RTE lettuce were obtained from a local factory belonging to a leading company in the market for processed food. The industrial processing included reception of raw material (*Lactuca sativa* var. *capitata* cv. *"Alpha"*), trimming, coring, chopping (approximately 3 × 3 cm), washing in a sodium hypochlorite and citric acid solution (oxidation reduction potential of 850–900 mV at pH 6.5–7.0) for 2 min, centrifuging (400 rpm for 60 s), weighting (300 g) and storing under active modified-atmosphere packaging (85–90% of N_2) in 70 μ co-extruded film (oxygen transmission rate < 5.000 cm^3/m^2*day*bar). Immediately after processing, the product was transported to the laboratory in coolers. The samples were stored at 4C ($\pm$1C) for 16 days. The whole experiment was repeated twice. In the first round, the sampling times were 1, 5, 8, 11, 13 and 15 days; and in the repeat experiment, the sampling times were 1, 4, 8, 11, 14 and 16 days. At each sampling time, four 300-g bags were sampled.

Ethical Considerations

The factory guaranteed the safety of its products based on prevention strategies through HACCP and Good Manufacturing Practices programs. To evaluate the safety of the product previous to the experiment, tests for specific pathogens (*Escherichia coli*, *Salmonella* and *Staphylococcus aureus*) and aerobic plate counts were carried out. The Ethical Committee of the "Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile" concluded that all samples were acceptable for human testing in the quantities to be served.

Consumer Study

Consumers who had eaten lettuce in the last week were recruited among students and personnel of our University in the city of Santiago, Chile; their ages ranged from 19 to 50 years. For each sampling time, 50–52 consumers were recruited to taste one sample. The sample consisted of approximately 25 g of lettuce presented in a covered transparent plastic Petri dish. The consumers were instructed to evaluate the appearance, texture and flavor of the lettuce and were asked to answer the following question with a "Yes" or a "No": "Would you regularly consume this lettuce?"

Survival Analysis

In food shelf-life studies, samples with different storage times are presented to consumers. Assume that we define a random variable T as the storage time at which the consumer rejects the sample. The rejection function $F(t)$ can be defined as the probability of a consumer rejecting a product before time t, i.e., $F(t) = P(T \leq t)$.

Storage times for the lettuce samples were between 1 and 16 days. If, for example, a consumer evaluated a sample stored for 11 days and rejected it, as it was the only sample evaluated by that consumer, then what could be said about his or her rejection time, was that it was less than 11 days. The data for this consumer were left-censored. If, on the other hand, a consumer accepted the sample stored for 11 days, what could be said about his or her rejection time, was that it was greater than 11 days. The data for this consumer were right-censored. The data set for each repetition of the experiment consisted of approximately 300 consumers with their corresponding storage times (50–52 consumers × 6 storage times), accompanied by a categorical variable indicating whether the time was left or right-censored. This type of data is referred to as current-status (Shiboski 1998) or quantal-response data (Meeker and Escobar 1998).

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

$$L = \prod_{\text{ifR}} (1 - F(r_i)) \prod_{\text{iff}} F(l_i) \tag{1}$$

where r is the set of right-censored observations and l is the set of left-censored observations. Equation (1) shows how each type of censoring contributes differently to the likelihood function.

Usually, rejection times are not normally distributed; instead their distribution is often right-skewed. For the evaluation of rejection times, a log-linear model is usually chosen:

$$Y = \ln(T) = \mu + \sigma W$$

where W is the error term distribution. That is, instead of the rejection time T, its logarithmic transformation is modeled. In Klein and Moeschberger (1997), different possible distributions for T are presented, e.g., the log-normal or the Weibull distribution. Choosing the Weibull distribution, the rejection function is equal to

$$F(t) = F_{sep}\left(\frac{\ln(t) - \mu}{\sigma}\right) \tag{2}$$

where $F_{sev}(\cdot)$ is the rejection function of the smallest extreme value distribution: $F_{sev}(w) = 1 - \exp(-e^{w})$, and μ and σ are the model's parameters.

The parameters of the log-linear model are obtained by maximizing the likelihood function (Eq. 1). The likelihood function is a mathematical expression that describes the joint probability of obtaining the data actually observed on the subjects in the study as a function of the unknown parameters of the model being considered. To estimate μ and σ for the log-normal or the Weibull distribution, the likelihood function is maximized by substituting $F(t)$ in Eq. (1) by the expression given in Eq. (2) if the Weibull distribution is chosen.

Visual assessment of how parametric models adjust to the nonparametric estimation was used to choose the most adequate model. For the present data, the following standard distributions were compared: smallest extreme value, normal, logistic, Weibull, log-normal and log-logistic. Details about each one of these distributions can be found in the literature (Klein and Moeschberger 1997; Meeker and Escobar 1998).

The repetition effect was analyzed with the following log-linear regression model with inclusion of covariates (Meeker and Escobar 1998):

$$\ln(T) = \mu + \sigma W = \beta_0 + \beta_1 Y + \sigma W \tag{3}$$

where T = time at which the consumer rejects the sample,

β_0 and β_1 = the regression coefficients,

Y = covariate indicating the first ($Y = 0$) or second ($Y = 1$) repetition,

μ = model parameter (for the Weibull model, see Eq. 2),

σ = model parameter (for the Weibull model, see Eq. 2), which does no depend on the covariate, and

W = error distribution.

Once the likelihood function is formed for a given model, specialized software can be used to estimate the parameters (μ and σ) that maximize the likelihood function for the given experimental data. The CensorReg procedures from S-PLUS (Insightful Corporation, Seattle, WA) were used to estimate the models' parameters, quantiles and corresponding standard deviations.

RESULTS

The Weibull distribution adjusted just as well as the other tested distributions, and being a flexible model for survival data, it was chosen to model $F(t)$. The repetition effect analyzed by Eq. (3) was not significant; thus, data from both repetitions could be grouped. This was verified graphically by showing that the rejection probability versus storage time curves for both repetitions overlapped (graph not shown). The parameters of the Weibull model (see Eq. 2) for the grouped data ± 95% confidence intervals were $\mu = 2.88 \pm 0.11$ and $\sigma = 0.37 \pm 0.09$. Using these parameters, the rejection function $F(t)$ can be plotted as shown in Fig. 1. Also in Fig. 1 are the nonparametric probability estimates (Meeker and Escobar 1998) showing that the Weibull model is a good fit to the data.

To estimate shelf life, the probability of a consumer rejecting a product, i.e., $F(t)$, must be chosen. Gacula and Singh (1984) mentioned a nominal shelf-life value considering 50% rejection, and Cardelli and Labuza (2001) used this criterion in calculating the shelf-life of coffee. Curia *et al.* (2005) calculated the shelf life of yogurt considering $F(t)$ values of 25 and 50%; these values were adopted in the present work. The estimated shelf lives are in

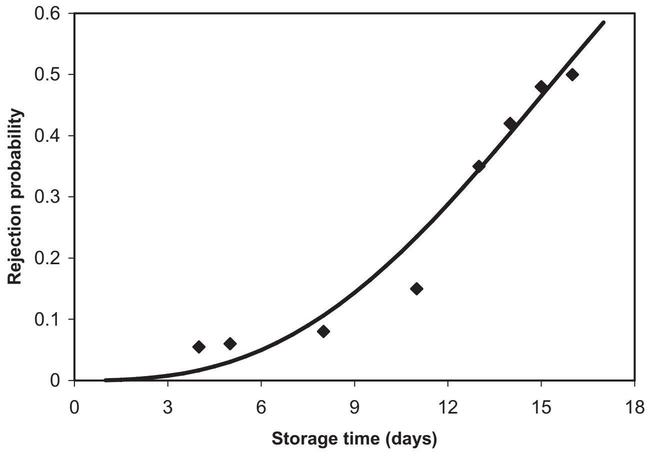

FIG. 1. CONSUMER REJECTION PROBABILITY AS A FUNCTION OF STORAGE TIME
Line is Weibull model and points are nonparametric estimates.

TABLE 1.
SHELF-LIFE VALUES ESTIMATED FOR 25 AND 50% PROBABILITY OF REJECTION BY CONSUMERS

Repetition	Shelf life (days) ± 95% confidence interval	
	For 25% rejection	For 50% rejection
I	11.3 ± 1.5	15.4 ± 1.2
II	11.1 ± 1.8	15.6 ± 1.4
I + II	11.3 ± 1.2	15.5 ± 0.9

Table 1. After duplication of the experiment, the most reliable shelf-life estimates were obtained from the grouped data. In practice, there may not be sufficient resources to perform a duplicate experiment; as shown in Table 1 the shelf-life estimates considering each replicate separately were similar to the grouped data, with slightly wider confidence intervals.

The commercial practice in Chile is to stamp a sell-by date of 10 days for RTE lettuce. Introducing this value in the model gave an estimated rejection probability ±95% confidence interval of 19 ± 7%. This can be considered acceptable; thus, the commercially used shelf-life is adequate.

CONCLUSION

As in previous studies (Hough *et al.* 2003; Curia *et al.* 2005), the focus of the shelf life has been set on the probability of a consumer rejecting a product after a certain storage time. In situations where it is not possible to have consumers taste all samples with different storage times in a single session, or when it is cumbersome to assemble the same group of consumers repeatedly for each storage time, it is advantageous to have a method where each consumer evaluates a single sample corresponding to a single storage time. Data were obtained from consumers using this methodology and survival analysis statistics were used to analyze results. These were satisfactory, showing that the methodology can be applied when necessary. A potential drawback of the method is that, as each consumer evaluates a single sample, a relatively large number of consumers are necessary. In the present work, 50 consumers per storage time lead to reliable results. It is good to remember that the number of consumers may be large, but their task is very simple: they have to try a single sample and decide whether they accept or reject it. Recommendations for the number of consumers and storage times necessary for desired statistical significance and power is the subject of our current research.

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