Determination of optimum ripening time in hard cooked cheeses (Reggianito type) using survival analysis statistics: Modified versus traditional cheese making technology

Garitta Lorena, Vélez Ayelén, Rodríguez Graciela, Perotti M. Cristina, and Hynes Erica

Abstract: In this work, we determined optimum ripening time of hard cooked cheeses made by traditional technology or by an innovative process aimed at accelerating flavor formation. For that purpose, we applied survival analysis statistics. Experimental cheese making (E) included homogenization of milk fat, unpasteurized cheese milk, changes in cooking temperature, and a curd-washing step, while traditional cheese making (T) followed a classic hard-cooked cheese making. Cheeses were ripened for 215 days and samples were analyzed at 76, 112, 128, 152, and 215 days. Consumers (250) were recruited and divided into five groups of 50 consumers for each stage. At each sampling time, consumers assessed whether the sample was "under-ripe," "ok," or "over-ripe." Optimum ripening time could be estimated only for E cheeses, with a high percentage of rejection. For T cheeses, it was not possible to determine the optimum ripening time because the rejection by over-ripening was never reported. We verified consumer segmentation: a small percentage found E cheese under-ripe and a high percentage found it over-ripe. Many consumers qualified E cheeses as too spicy, especially at the end of ripening. Spicy flavor is usually perceived before than the texture and evidenced an acceleration of the flavor formation. We concluded that the innovative intervention in cheese making technology was successful in accelerating cheese ripening. It also had potential to develop a new cheese product targeted at consumers who chose/prefer good spicy flavor.

Keywords: cheeses, ripening, survival analysis, innovative technology

Practical Application: Survival analysis is a useful methodology to determine the optimum ripening time of foods based on consumer data. In this work, it evidenced that the proposed innovative cheese making was successful in accelerating the formation of cheese flavor, and had the potential to develop a new cheese product targeted at consumers who chose/prefer good spicy flavor.

1. INTRODUCTION

Cheese ripening comprises a complex set of physical and biochemical changes, involving the metabolism of residual lactose, citrate and lactate, proteolysis and lipolysis, and catabolism of amino acids and free fatty acids. During ripening, the curd is transformed in a mature cheese with distinctive characteristics of texture, aroma, and flavor (Fox & McSweeney, 2017).

Reggianito is an Argentinean cheese that was introduced by Italian immigrants in the 19th century. It was inspired by Italian hard cheeses such, as Grana Padano and Parmiggiano Regiano, but the cheese making was modified and adapted to available raw materials and environmental conditions to give a distinctive product (Candioti et al., 2002; Zannoni et al., 1984). As other hard cooked cheeses, it requires at least 6 months of ripening to develop appropriate sensory characteristics (Vélez et al., 2010).

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The main disadvantage of long ripening times consists of the costs, due to significant capital immobilized. Therefore, acceleration of cheese ripening interests highly to cheese industry and is a hot topic of research (Khattab et al., 2019). Ripening also provides an opportunity to diversify products; that is, Cheddar cheese is marketed as "mild," "cured or sharp," "extra sharp", these terms identified in the packaging indicate the ripening time and are related to sensory characteristics. Typically, the oldest Cheddar cheeses have the most intense flavor, dry and crumbly texture, and can be difficult to melt, while mild Cheddar cheeses are softer, more elastic, smoother, and easier to melt.

Several authors have proposed technological changes in cheese making or ripening of hard cooked cheeses (Reggianito type) and assessed their effect, aiming at ripening acceleration (Hough et al., 1994; Hynes et al., 2003; Perotti et al., 2004, 2005; Sihufe et al., 2010; Vélez et al., 2010, 2019, among others). However, few studies included sensory analysis (Hough et al., 1994, 1996; Sihufe et al., 2010; Vélez et al., 2019). There is no previous report on consumer's response to estimate the performance of technological changes on ripening acceleration or the optimum ripening time (ORT).

Many methodologies have been proposed to evaluate the sensory attributes of cheese, which can be classified into two broad

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categories: conventional sensory methodologies, such as descriptive profiling analysis, conducted by trained assessors (Kraggerud et al., 2008), and other methodologies performed by untrained consumers namely check-all-that-apply (CATA), acceptability testing, triangle test, projective mapping, and flash profiling or napping (De Moraes et al., 2018; Gkatzionis et al., 2013; Queiroga et al., 2013; Sanches-Macias et al., 2012; Valentin et al., 2012).

Survival analysis (Klein & Moeschberger, 1997; Meeker & Escobar, 1998) is a branch of statistic methods used extensively in clinical studies, epidemiology, biology, sociology, and reliability studies. In this method, the failure function F(x) can be defined as the probability of an individual failing before time "x" (Klein & Moeschberger, 1997). Hough et al. (2003) applied survival analysis to sensory shelf-life of foods. In this context, the "individual" would not be the food itself, but rather the consumer; therefore, the failure function would be defined as the probability of a consumer rejecting a product stored for a time lower than "x." The risk would not be focused on the product deteriorating but rather on the consumer rejecting the product. Garitta et al. (2006) developed a model to estimate optimum concentrations of a food additive (coloring) using survival analysis. They applied the model to the red color in strawberry yogurt, where the color can be too light, ok, or too dark, leading to two events of interest: the transition from too light to ok, and the transition from ok to too dark. Therefore, we proposed to apply a similar scenario to cheese ripening.

Vélez et al. (2019) proposed an innovative cheese making procedure for hard cooked cheese aimed at accelerating the formation of characteristic flavors, thus, reducing the storage time compared with the traditional technology. The intervention included the homogenization of milk fat, no thermal treatment of cheese milk, cooking temperature modification, and the inclusion of a curd-washing step. They assessed the influence of these changes on the biochemistry of ripening and on sensory profile performed by a trained panel. Ripening acceleration was observed in cheeses made with the modified technology based on an increase in lipolysis and proteolysis reactions. Moreover, the formation of some key volatile compounds derived from fat degradation reached the maximum levels at 45 days. These results were in agreement with the sensorial analysis, as an increase of spicy taste and an overall intensification of flavor were detected; no atypical flavors were detected. Information on consumers' response was, however, lacking to complete this research.

In this work, we applied survival statistics tools based on consumer's response to hard cooked cheeses obtained from both technologies to determine the ORT.

2. MATERIALS AND METHODS

2.1 Cheese samples

Hard cooked cheeses from innovative and traditional cheese making technology were compared. Experimental (E) and traditional (T) processes were conducted in parallel vats as reported by Vélez et al. (2019). E and T cheese milk (2.5% fat) was prepared by mixing homogenized or unhomogenized cream (9 MPa/45 °C, Gaulin Homogenizer 31M-3 TA, SPX Flow, Inc., Charlotte, NC, USA), respectively, with skimmed milk. For E cheeses, milk was maintained at 33 °C without pasteurization. For T cheeses, milk was pasteurized at 63 °C for 20 min and then cooled to 33 °C. In both cases, $CaCl_2$ (Merck, Darmstadt, Germany) (0.014% w/v) and lactic acid (15 g/L) were added up to target pH (6.3 to 6.4). Starter was added equally to both vats (*Lactobacillus helveticus* LH-B02 and *Lactobacillus bulgaricus* Lb-12, Chr. Hansen, Denmark) at 10^6 CFU/mL of milk. Then, coagulant (0.62 g/50 mL of distilled water, Maxiren 150, France) was added per vat. When coagulum acquired adequate firmness, it was cut until the grains were approximately the size of a grain of rice (length of 6 to 7 mm and 2 to 3 mm in width). The mixture curd grains-whey was heated under stirring. For both types of cheese, heating (1 °C/min) proceeded until 45 °C. At this moment, a portion of whey (30 L) was replaced by a lactose solution (4.5% w/v at 45 °C) in E cheeses. Then, E and T mixtures were heated at 50 and 52 °C (<1 °C/min), respectively. Finally, stirring was stopped, curds were put into molds and pressed for 12 hours, brined for 3 days (saturated brine at 12 °C).

Cheeses (approx. 3.5 kg) were ripened for 215 days at 12 °C and 80% relative humidity and samples were analyzed for sensory attributes at 76, 112, 128, 152, and 215 days. These sampling times were selected according to the results obtained previously by a trained panel. In fact, cheeses were tested at each 10 days by a panel of six trained assessors to confirm sensory changes.

Products were not tested before 76 days since the Argentinean Legislation does not allow the intake of cheeses made with raw milk ripened at a temperature higher than 5 °C for a period of less than or equal to 60 days (CAA, 2020).

Both cheeses had similar ($p > 0.05$) physicochemical composition determined by standard methods (at 76 days of ripening): moisture (%) (ISO, 2004), 38.5 ± 1.0 and 41.5 ± 1.3; fat in dry matter (%) (ISO, 2008), 41.0 ± 1.0 and 43.2 ± 0.5; protein in dry matter (%) (ISO, 2011), 48.0 ± 1.0 and 49.2 ± 2.0, and pH (Bradley et al., 1992), 5.45 ± 0.04 and 5.47 ± 0.03, for T and E cheeses, respectively.

2.2 Consumer study

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. For this, a reverse storage design is necessary (Hough et al., 2006). This type of design is not always appropriate. An alternative is to let the same consumers perform repeated tests for each one of the storage times (Garitta et al., 2008). Assembling the same group of consumers on six or seven occasions corresponding to each storage time can be cumbersome and costly. In addition, these consumers soon realize that they are participating in a shelf-life study and this introduces an expectation error, whereby they feel they must start rejecting samples at some point. Another issue with consumers tasting several samples in a single session is how representative this situation is of real consumption. It would be more representative to collect information of consumers after they take home a product, for example, a single bottle of beer with an established storage time. Araneda et al. (2008) developed a method for predicting sensory shelf-life for situations in which each consumer evaluates only one sample corresponding to one storage time, thus, avoiding the problem of consumers having to evaluate multiple samples corresponding to multiple storage times. This type of data is known as current-status data (CSD) in survival analysis statistics (Shiboski, 1998).

In the present work, CSD was applied for the study design. Thus, each consumer tasted a single sample corresponding to a single ripeness stage. Therefore, for each sampling time, consumers tasted only one cheese T sample and only one cheese E sample.

The number of consumers necessary for survival analysis estimations based on each consumer evaluating a single sample was calculated following the guidelines of Libertino et al. (2011). Thus, 250 consumers aged from 25 to 60 years were recruited from the city of 9 de Julio (Buenos Aires, Argentina). They were regular consumers (at least once a week) of hard cheeses and all of them were divided into five groups of 50 consumers for each sampling time. Each group was composed by different people. The tests were conducted at the facilities of our Institute. The study was approved by the Instituto Superior Experimental de Tecnología Alimentaria (ISETA) and consent was obtained from each subject before their participation in the study.

Samples of E and T cheese cubes of 1 cm side were cut from the cheese center, discarding the edges, placed on a white plate coded with a random three-digit number. For each sample, the consumer had to assess whether the sample was "under-ripe" (undeveloped flavor/not spicy/soft), "ok" (characteristic flavor), or "over-ripe" (highly developed flavor/extremely spicy/hard).

2.3 Statistical analysis

The model is basically the same as the one developed by Garitta et al. (2006) for determining the optimum color of yogurt. In that model the explanatory variable was instrumental color, while here it is ripeness time (RT).

Let RT be the random variable representing the optimum RT of a cheese for a consumer. Assume that RT follows the cumulative distribution function F. For each value of ripeness time (rt), there will be two rejection functions:

- R_u (rt) = probability of a consumer (or proportion of consumers) rejecting a cheese at a time > rt because it is under-ripe, that is, R_u(rt) = P (RT > rt) = 1 - F(rt)
- R_o (rt) = probability of a consumer (or proportion of consumers) rejecting a cheese at a time < rt because it is over-ripe, that is, R_o(rt) = P (RT < rt) = F(rt)

Usually, survival times are not normally distributed; instead, their distribution is often right skewed. A log-linear model is used:

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

where μ = expected mean of ln(RT), σ = standard deviation, and w is the error term distribution. That is, instead of the survival time RT, its logarithmic transformation is modeled. In Klein and Moescheberger (1997) or Lindsey (1998), different possible distributions for RT are presented, for example, the log-normal or the Weibull distribution. Choosing the Weibull distribution, the functions associated with the rejection are:

$$R_u(rt) = 1 - \exp\left[-\exp\left(\frac{\ln(rt) - \mu_u}{\sigma_u}\right)\right] \quad (1a)$$

$$R_o(rt) = \exp\left[-\exp\left(\frac{\ln(rt) - \mu_o}{\sigma_o}\right)\right] \quad (1b)$$

In Eq. 1a and 1b, exp [-exp] is the distribution function of the smallest extreme value distribution, and μ_u, μ_o, σ_u, and σ_o are the model´s parameters.

Instead, if the Log-normal distribution is chosen, the functions associated with the rejection are:

$$R_u(rt) = 1 - \Phi\left(\frac{\ln(rt) - \mu_u}{\sigma_u}\right) \quad (2a)$$

$$R_o(rt) = \Phi\left(\frac{\ln(rt) - \mu_o}{\sigma_o}\right) \quad (2b)$$

where, for both events, Φ (.) is the cumulative normal distribution function and μ_u, μ_o, σ_u, and σ_o are the model's parameters.

To analyze if there are differences in the ripening time between technologies (E and T cheeses), regression models with the inclusion of covariates were applied (Klein & Moescheberger, 1997; Meeker & Escobar, 1998). The complete models for each event are as follows:

Model for rejection due to under-ripe:

$$Y = ln(rt_u) = \mu_u + \sigma_u W = \beta_{0u} + \beta_{1u} Z_u + \sigma_u W \quad (3a)$$

where

rtu is the ripening time at which a consumer rejects a sample because it is under-ripe, β_{0u} and β_{1u} are the regression coefficients, Z_u is covariate indicating if sample cheese is T (Z = 0) or E (Z = 1), σ_u is shape parameter, which does not depend on the covariates, and W is the error distribution.

Model for rejection due to over-ripe:

$$Y = ln(rt_o) = \mu_o + \sigma_o W = \beta_{0o} + \beta_{1o} Z_o + \sigma_o W \quad (3b)$$

where

rt_o is the ripening time at which a consumer rejects a sample because it is over-ripe, β_{0o} and β_{1o} are the regression coefficients, Z_o is the covariate indicating if sample cheese is T (Z = 0) or E (Z = 1), σ_o is the shape parameter, which does not depend on the covariates, and W is the error distribution.

ORT was obtained from the minimum of the curve that resulted from adding the curve of rejection due to under-ripe and the curve of rejection due to over-ripe (Hough, 2010). The formula for calculating ORT's confidence interval has been published previously (Garitta et al., 2006).

To define the distribution models, which best adjusted the experimental data (normal, log-normal, or Weibull distributions), the loglikelihood criterion was used (Hough, 2010). Survival analysis calculations were performed with the TIBCO Spotfire S+ software (TIBCO, Inc., Seattle, WA).

3. RESULTS AND DISCUSSION

The consumer data for the event "under-ripe" were best adjusted by the Weibull distribution for both cheeses (T and E). For the "over-ripe" event, the best-fitting distribution was log-normal both for T and E samples. When in the model the technology covariate was included following Eq. 3a and 3b, only for "over-ripe" event the covariate was significant.

Thus, the model´s parameters were:

Event "under-ripe" (nonsignificant covariate)
T and E cheeses, μ_u: 4.91
σ_u: 0.44
Event "over-ripe" (significant covariate)
T cheeses, μ_o: 6.45
E cheeses, μ_o: 5.46
σ_o: 0.79

The rejection curves, made with parameters, for both events are presented in Figures 1 and 2.

Figure 2 shows a percentage of rejection less than 10% for traditional cheeses. It means that consumers did not consider T cheese sample over-ripe, even in the last stage (215 days). As mentioned, Reggianito cheese requires at least 6 months of ripening for the sensory characteristics to develop properly. Therefore, the consumers' response obtained was logical and in accordance since

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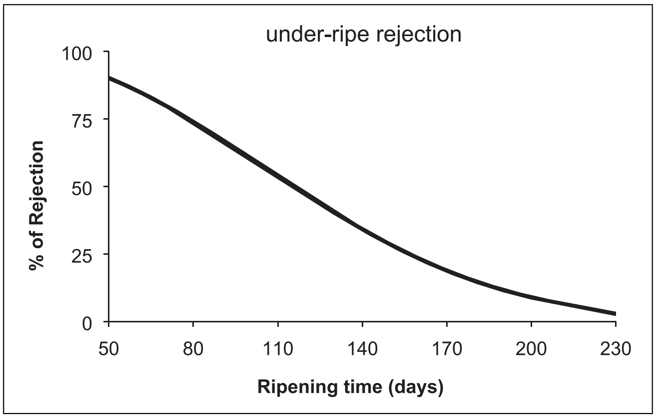

Figure 1–Percentage of rejection for under-ripe event.

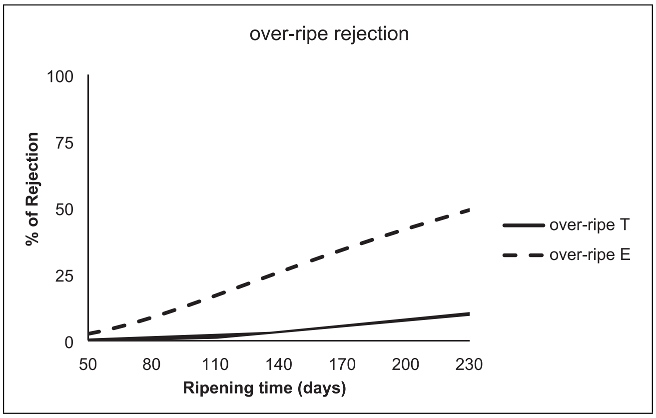

Figure 2–Percentage of rejection for over-ripe event, considering traditional (T) and experimental (E) cheeses.

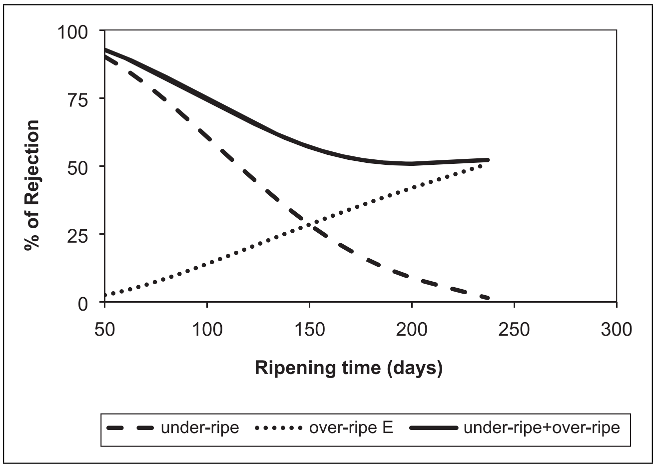

Figure 3–Optimum ripening time (sum of under-ripe rejection + over-ripe rejection).

cheeses with approximately 7 months of maturation did not reach the expected ripeness.

Then, the small percentage (10%) made that when adding both rejection curves to estimate the ORT, the minimum value necessary for the calculation could not be obtained. Only the ORT for the experimental cheese could be estimated. The curve resulting from the sum of under-ripe and over-ripe curves is presented in Figure 3. The estimated ORT and its confidence intervals were 200 ± 22 days. The ORT obtained corresponds to approximately six and a half months of ripeness; this time is similar to the minimum required by the cheese industry when traditional technology is applied. At this optimum time, there was still a high percentage of rejection (50%): 9% of consumers found these samples under-ripe, and 41% found them over-ripe.

This scenario is similar to that found in the study by Garitta et al. (2006) in which they modeled the color intensity of a yogurt. They concluded that there would not be an ideal color of the product on its own that would be considered acceptable by all consumers. While one consumer found it too light, another found it too dark and vice versa. Therefore, the next step was to find an optimum color that maximizes the proportion of consumers for whom the color is appropriate. At this optimum, there will be a group of consumers who will find it appropriate, another group who will find it too light, and yet another group who will find it too dark. A high percentage of rejection at the optimum over 20% was found. Similar results were informed for optimization of salt concentration in bread (Sosa et al., 2008), cooking temperature of beef (López Osornio et al., 2008), and sucrose concentration in probiotic petit Suisse cheese (Esmerino et al., 2015).

During this work, many consumers mentioned that cheese was too spicy, especially in the last stage. For E cheeses, the development of the flavor and texture might not have occurred simultaneously; spicy flavor was perceived before the texture, showing an acceleration of the flavor formation process. Results perceived by consumers agree with those found by the trained panel as mentioned in Velez et al. (2019); in particular, at 45 days E sample had more total intensity of aroma and spicy aroma, and at 60 days E cheese had greater intensity flavor than T cheeses. In addition, at 60 days sensory characteristics of E cheeses were associated with higher values of proteolysis and peptidolysis when compared to T cheeses: SN pH 4.6/TN, 15.6 versus 13.2; SN TCA/TN, 14.0 versus 10.5 and SN PTA/TN, 8.3 versus 5.6, respectively. Lipolysis degree (μmoL/100 g fat) was also higher in E than T cheeses: 25,000 versus 4,000, respectively. These results were probably due to the use of raw milk and lower cooking temperature applied in E cheeses, as similar results have been reported (Bachmann et al., 2011; Grappin & Beuvier, 1997; Vélez et al., 2016).

According to the results arrived in this work, two segments of consumers can be distinguished: consumers who like cheeses in the under-ripened range and consumers who like cheeses in the over-ripened range. Therefore, the innovative cheese product can be appropriate for the segment of consumers who choose a cheese with good spicy flavor. To confirm this, we suggest that for further studies, the segmentation of the consumers may be considered. According to Garitta et al. (2006), judgments conducted using survival analysis are considered to show greater agreement with the reality of consumer choices. Similar results were found in the ORT of tomatoes (Garitta et al., 2008), where two segment consumers were found: consumers who liked the fruits in the under-ripened range and consumers who liked the fruits in the over-ripened range. In the Argentinean market, products are targeted to segmented consumer groups, for example, "regular" and "hot" ketchup, or "regular" and "extra strong" mint sweets (Garitta et al., 2006).

As mentioned before, Cheddar cheese is marketed as sharp, mild, extra sharp in the United States and Ireland, for example. Our results, consisting of acceptance or rejection data obtained from consumers to satisfy the preferences of different segments, offer the potential of diversifying Reggianito cheeses by defining ORT for specific consumer groups.

The information obtained from consumers using survival method is related to their everyday eating/buying routine and, thus, is relevant for product marketing or development. In survival analysis, only responses related to consumption, limited to the answers yes or no, are requested (Hough et al., 2003; Hough et al., 2006; Garitta et al., 2018). Unlike in sensory profiling method, no pressure is exerted on consumers to assess the intensity of an attribute. The analysis is perceived as familiar and commonsense by the consumers because making decisions is part of their consumption, which in turn makes results more reliable. Thus, survival analysis may represent more clearly the expectations of consumers with respect to a product. This methodology taps directly into consumer experience (Esmerino et al., 2015; Garitta & Hough, 2012a; Garitta et al., 2015).

Survival analysis has shown potential as an advantageous tool to be applied to various foods and ingredients in dairy companies. In fact, Hough et al. (2004) determined consumer acceptance limits to sensory defects in ultra-high temperature milk using survival analysis. Curia et al. (2005) applied this method to estimate the sensory shelf-life of commercial Argentine stirred yogurt of different compositions. Garitta et al. (2006) estimate the optimum color in liquid strawberry yogurt using survival analysis statistics. Cruz et al. (2010) applied survival analysis methodology to predict the shelf-life of probiotic flavored yogurt. Esmerino et al. (2015), used this method to estimate the optimum sucrose concentration in strawberry-flavored probiotic petit Suisse. Garitta et al. (2015) applied interval-censored survival analysis techniques to estimate sensory cut-off points based on consumer's decision to accept or reject food products considering the inherent variability in sensory measurements (acid flavor in yogurt).

The main disadvantages of this method are that specialized statistical software is necessary and, when there are many variation factors in the experiment, a high number of consumers is required (Garitta & Hough, 2012b). The choice of the time periods of the study needs special attention. The fact that many consumers did not consider traditional cheeses "over-ripe" at the last sampling point may indicate that the time window was too short. For fruits, for example, Garitta et al. (2008) found that several consumers accepted the samples at the chosen initial time; it does not indicate that these consumers have odd choices; it indicates that the study began too late. In future works, the choice of time periods is an issue to be dealt. The market sampling is another topic to consider because if the sampling of the target market was not done properly, it could end up with inaccurate consumer preference data.

4. CONCLUSION

In this work, we applied survival analysis methodology to determine the ORT of hard cooked cheeses made by an innovative technology and by the traditional cheese making procedure, based on consumer data. The sensory analysis applied was relatively simple; only 50 consumers had to decide at each ripening time if they found the samples under-ripe, ok, or over-ripe. This censored data set was sufficient to determine the ORT of the experimental cheese obtained from the intervention, but not for traditional cheeses as rejection by over-ripe was never produced.

The optimum time assessed for experimental cheese was up to six and a half months, which represented an acceleration of ripening, specifically of flavor formation. Consumers´ segmentation was found: a small percentage perceived this cheese under-ripe, but a high percentage found it over-ripe. These results offer the potential of diversifying Reggianito cheeses by defining ORT for specific consumer groups.

Finally, it is important to consider that more research is needed to determine the optimal expiration time of the products, as flavor development continues over time depending on the storage temperature.

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AUTHOR CONTRIBUTIONS

Dr. Garitta designed the study, performed statistical analysis, interpreted the results, and drafted the manuscript. Drs. Veléz, Perotti, and Hynes designed the new cheese making technology, performed chemical analysis, and collaborated with the drafting the manuscript. Lic. Rodríguez conducted the consumer study and collected test data. All authors have approved the final article.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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