DAIRY FOODS

Sensory and Microbiological Shelf-Life of a Commercial Ricotta Cheese

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ABSTRACT

Ricotta is a soft cheese that has a high moisture content and an initial pH above 6.0 and, thus, has a limited shelf-life even under refrigeration. The objectives of the present work were to calculate sensory and microbiological shelf-life using Weibull's distribution and to obtain kinetic parameters to predict shelf-life for different storage temperatures. Ricotta cheese was stored at 6, 17, and 25°C; during storage, samples were removed for sensory, microbiological, acidity and pH analyses. Appearance, texture, flavor, total aerobic mesophiles, and acidity followed a similar pattern over storage time. As expected, pH decreased over storage time, although the changes were irregular. Shelf-life values at ±95% confidence limits, calculated from Weibull's distribution, were 33 ± 1.4, 12.5 ± 0.5, and 5.5 ± 0.5 d for temperatures of 6, 17, and 25°C, respectively. Q_{10} (reaction rate at T + 10°C/reaction rate at T°C) for shelf life was 2.52 between 6 and 16°C and the corresponding activation energy was 14.8 kcal/mol.
(**Key words**: cheese, Ricotta, shelf-life prediction, sensory)

Abbreviation key: **F(x)** = probability of product failure, **H(x)** = cumulative hazard, **x** = days of storage, $\mathbf{Q_{10}}$ = reaction rate at T + 10°C/reaction rate at T°C.

INTRODUCTION

Ricotta is a soft Italian cheese that is popular in Italy and Ibero-American countries such as Argentina. It can be produced using cheese whey, or milk, or a mixture of both. If made from whole milk, it is soft and creamy with a delicate texture and pleasant, slightly caramel flavor (15). Argentine legislation indicates it must have a maximum of 75% water and 11 to 13% fat if made from whole milk (5).

Ricotta has a high moisture content, and its initial pH is above 6.0 (15), thus it is susceptible to microbial spoilage, and, even under refrigeration, it has a limited shelf-life. There are no reports in literature on the shelf-life of this product. For cottage cheese, similar in composition to Ricotta, Labuza (16) reported a shelf-life of 14 d at 0 to 2°C and Muir (19) mentioned factors affecting shelf-life of this product without presenting data. There are no reports for activation energy or Q_{10} values for this type of dairy product. In Argentina, the "eat-before" date stamped on the package of commercial Ricotta is between 24 and 30 d under refrigeration as reported by the two leading manufacturers (Nestlé Argentina Buenos Aires, Argentina and Unión Gandarense Buenos Aires, Argentina). These manufacturers informed us that these "eat-before" dates are based on commercial experience.

Few studies (11, 23, 24) have used statistical distributions to model the shelf-life of foods. The probability of reaching the calculated shelf-life and the corresponding confidence intervals can be calculated using these distributions. Gacula and Kubala (9) and Gacula and Singh (10) discussed the use of the normal, log-normal, and Weibull distributions for modeling the shelf-life of foods. They conclude that the Weibull distribution is the most adequate.

When performing shelf-life studies, different sensory attributes have been used: off-odor or flavor (7), overall acceptability (4), quality (20) and deviations of typical flavor as compared with a reference (1). These attributes can be subjective. For example, one assessor can rate a sample as poor in quality because it differs from fresh, and another can consider the same sample as good quality because the differences from fresh do not affect his or her acceptability. Similarly off-odors or flavors are difficult to define, particularly in shelf-life studies in which unexpected sensory changes are likely to confuse assessors trained to detect a specific off-odor or flavor. A more objective approach would be to use a difference-from-control test (17) with assessors measuring overall differences and not a specific descriptor.

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The objectives of the present work were 1) to analyze the relationship between sensory and microbiological changes during the storage of a high moisture cheese such as Ricotta, 2) to apply the Weibull distribution to calculate the sensory and microbiological shelf-life of Ricotta cheese, and 3) to obtain kinetic parameters to predict shelf-life as a function of storage temperature.

MATERIALS AND METHODS

Ricotta Cheese

Ricotta cheese was from pasteurized whole cow's milk provided by Nestlé Argentina S.A. The manufacturing process was as follows: cheese whey was mixed with whole milk in a proportion of 40:60. The mixture was heated 30 to 32°C and a *Streptococcus thermophilus* starter was added, which increased the acidity to approximately 0.24% lactic acid. The temperature was increased to 85°C and was maintained for 20 min. Titrable acidity was adjusted to 0.3% lactic acid using lactic acid. The Ricotta cheese was collected in molds to partially eliminate whey. Then, the cheese was placed in a tank where it was stirred, and the final moisture was adjusted to 70%. Finally, it was packaged at temperatures between 65 and 68°C in 500-g polyethylene bags and then cooled to 5°C. The composition was provided by the company: protein, 14.9%; fat, 12.6%; carbohydrates, 2.7%; ash, 1.5%; and water, 68.3%. This composition is similar to that indicated by Kosikowski (15) for Ricotta cheese made from whole milk and is also similar to the composition of that from the other major Ricotta manufacturer in Argentina (Unión Gandarense, 1998, personal communication).

Storage Temperatures and Sampling Times

To study the effect of storage temperature, shelf-life experiments were conducted at 6, 17, and 25°C ± 0.5°C. The use of conventional designs in food taste testing experiments often requires a prohibitive size of samples because of the destructive nature of the sampling procedure. As Gacula (8) pointed out, the problem is to develop designs for shelf-life studies with a reasonable sample size. An approach to the problem is to concentrate the majority of samples in the experimental periods in which the maximum information is desired. This time is the period in which the samples are likely to fail or are approaching the limit of acceptable quality. Gacula (8) presented designs for shelf-life study that increased the number of samples in the period in which the product was likely to fail with the criteria of accelerating sampling when 50% of the products fail. For 6 and 17°C, we followed this criteria but not for 25°C as the shelf-life at this temperature was so short that we sampled daily. The resulting sampling times at each temperature were 6°C: 6, 13, 20, 27, 30, 33, 36, and 37 d; 17°C: 2, 5, 8, 11, 12, 13, and 14 d; and 25°C: 1 to 7 d.

At each sampling time, two bags were removed from the refrigerator or oven, and each was submitted to sensory, microbiological, and chemical analyses.

Sensory Analysis

A difference-from-control test (17) was used with a 7-point structured scale anchored as follows: 0 = no difference, 1 = very slight, 2 = slight, 3 = moderate, 4 = moderately big, 5 = big, and 6 = very big.

The fresh control samples were provided by the Nestlé Argentina and were kept at 2 to 3°C for a maximum of 7 d. Before replacing the control samples with fresh samples, a sensory triangle test using 15 trained assessors was performed to ensure that the fresh batch was not different ($P < 0.01$) from the previous batch.

The shelf-life panel consisted of 8 assessors that were trained in discrimination and in descriptive analyses of a number of products. For each sampling time, assessors received the following: 1) the control sample labeled as K; 2) two samples, one from each of the 2 bags that were removed from shelf-life storage and were labeled with 3-digit code numbers; and 3) a blind control that was labeled with a 3-digit code number.

The samples were served at room temperature. Assessors measured color, appearance, texture, and flavor. For color and appearance, the cheeses were placed in 5-cm diameter Petri dishes and were evaluated in a cabin with white fluorescent daylight-type illumination. For color, assessors had to measure the degree of difference from the control of the white-creamy color. Appearance was defined as all visual impressions other than color. For texture and flavor, 20 g of cheese were placed in a 100-ml glass and were presented to assessors, who were in individual booths. Texture was measured manually by stirring the sample with a spoon. One teaspoonful of cheese was used for flavor evaluation. Unsalted crackers and water were used for rinsing between samples.

Microbiological and Chemical Determinations

The following microorganisms were analyzed for the fresh cheese and at each of the storing times using standard techniques (13): total aerobic mesophiles, yeast and molds, psycrophiles, coliform, and *Staphylococcus aureus*. Titrable acidity and pH were measured by standard techniques (2).

Failure Criteria

Failure criteria in sensory testing are not uniform. Gacula (8) used different cut-off points according to the product or situation: on a scale where 1 = no off-flavor to 7 = very strong off-flavor, he used 2.5 for one product and 3.5 for another. Randell et al. (22) used a quality scale where 0 = unacceptable to 5 = excellent, and they considered products with scores ≤2 as unacceptable for sale and ≤1.5 unfit for human consumption. O'Conner-Shaw et al. (20) used qualitative appraisals such as lower typical odor. Other authors (23, 24) have asked consumers for yes-no answers as to whether or not they would consume the product.

From the just mentioned review, it is clear that there are no standards for defining sensory failure of food products. Ricotta cheese is a fresh dairy product, and consumers are not likely to tolerate any changes in color, texture, or flavor resulting from prolonged storage. Thus, we chose a score ≥1.5 (between very slight and slightly different from the fresh control) as the cut-off point to define failure. The sample score was obtained by subtracting the mean of the blind control from the mean of the sample. The use of the estimate obtained with the blind controls amounts to obtaining a measure of the placebo effect, which represents the numerical effect of asking the difference question, when, in fact, no difference exists (17).

Argentine food regulations specify microbiological standards for the generic category of cheeses with water content higher than 55%. These limits are >1000 coliforms/g, >500 *Staph. aureus*/g, and >5000 yeasts and molds/g. No limits are set for aerobic mesophiles. The limit for aerobic mesophiles is $>10^5$ cfu/ml in pasteurized whole milk and $>10^5$ cfu/g in cream and casein (5). Similar limits for aerobic mesophiles have been reported by other authors (6, 14, 18) for milk, cream, and ice cream. For the present work, we considered a limit of $>10^5$ cfu/g for total aerobic mesophiles as failure criteria.

Statistical Analysis

Weibull's distribution was used to model the shelf-life data (10). Calculations were performed with a specific software, which included the Kolmogorov-Smirnov goodness of fit criteria (3).

RESULTS

Figure 1 shows flavor, total aerobic mesophiles, acidity, and pH as a function of storage time for the three temperatures. The choice of making a cut-off point of 1.5 on a 0 to 6 difference from the control scale for flavor seems reasonable (Figure 1A) as it is approximately at this point that the product starts to change from a sensory point of view. The cut-off point of 10^5 cfu/g for microbial growth was considered in relation to sanitary recommendations for similar food products. Figure 1B shows that 10^5 cfu/g were reached at storage times similar to those corresponding to the sensory cut-off point shown in Figure 1A. Appearance and texture (not shown) evaluations were similar to those of flavor, although maximum values of difference from control were smaller in the range of 2.5 to 3 compared with 4.8 to 5.7 for flavor. The increase in titrable acidity (Figure 1C) followed a pattern similar to flavor and microbial growth. pH (Figure 1D) decreased as expected, although changes were irregular; at 17°C, the pH hardly changed over time, yet flavor and microbial changes were significant. We are unable to explain this irregular pH behavior.

Spearman's Rank Correlation test (21) was used to analyze the association between microbial growth versus flavor and acidity increases. For microbial growth versus flavor, the coefficients were 0.93, 0.93 and 0.77 for storage temperatures of 6, 17, and 25°C, respectively. For microbial growth versus acidity, the coefficients were 0.99, 0.99, and 0.97. All these coefficients were significant ($P < 0.01$, except for coefficient = 0.77 with $P < 0.05$), thus showing association between microbial growth versus flavor and acidity increases.

Color did not change over storage times, and scores were always below 1 on the 0 to 6 sensory scale. Coliforms, psychrophiles, and *Staphylococcus aureus* were always negative. The absence of these microorganisms was to be expected because of the use of pasteurized milk, high hygienic standards, and packaging temperatures above 65°C. Yeasts and molds had initial counts between 0 and 200 cfu/g and only grew at 25°C to 1500 cfu/g, which was below the failure criteria indicated earlier for this type of cheese.

In Table 1 are the shelf-lives calculated from Weibull's distribution considering a probability of finding a failed product of 50%. The Kolmogorov-Smirnov goodness of fit criteria (3) was reached in all cases.

The large confidence interval for appearance shelf-life at 6°C, was due to having only three samples reach the failure criteria for appearance at this temperature. Thus, the Weibull distribution was fitted for only three points.

In the present study, product failure was due to aerobic mesophile growth, which increased acidity and altered sensory properties. No health risk is associated with consuming this Ricotta cheese close to the end of its shelf-life; if there was a health risk, the probability of finding a failed product would have to be lower, 5 or 10%.

The last row of Table 1 was calculated under the assumption that a sample had failed when any one of its attributes were above the predefined cut-off points. Whether each attribute was considered individually or all as a group, similar values were obtained for shelf-life, which implies that sensory and microbial changes were simultaneous.

The equations relating days of storage (x), cumulative hazard [H(x)], and probability of product failure [**F(x)**] are as follows (9):

$$H(x) = (x/\alpha)^{\beta} \quad [1]$$

$$F(x) = 1 - \exp[-H(x)] \quad [2]$$

where α and β in Equations [1] and [2] = regression constants. The straight line obtained by least square

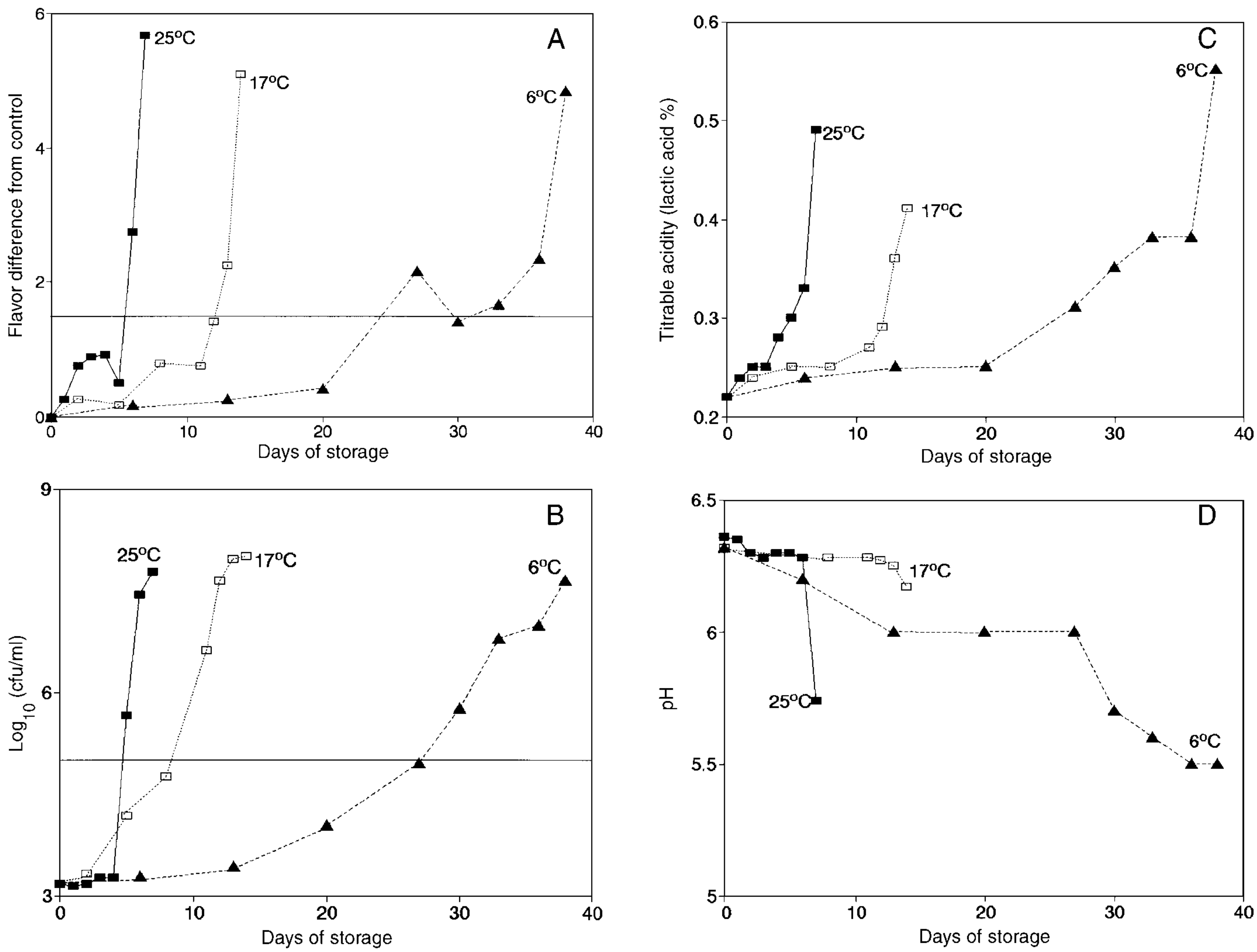

Figure 1. Changes in Ricotta cheese samples stored at 6, 17, or 25°C versus a fresh control. Flavor differences (A) were rated on a 7-point scale where 0 = no difference to 6 = very big difference. The horizontal line shows the cut-off point at a score of 1.5 (between very slight and slightly different from the fresh control) to define sample failure. Growth of aerobic mesophiles (B) with cut-off point indicated by horizontal line, titratable acidity (C), and pH variations (D) are also shown for the three temperatures over storage time.

TABLE 1. Days of shelf-life and 95% confidence intervals, calculated at 6, 17, and 25°C for aerobic mesophiles, appearance, texture, and flavor and for all attributes.[1]

	6°C		17°C		25°C	
Attribute	d	95% CI	d	95% CI	d	95% CI
Aerobic mesophiles	36	1	12.5	0.5	6.4	1
Appearance	37[2]	. . .	13.5	0.5	6.2	1.2
Texture	36	2	13.0	0.5	5.7	1
Flavor	33	1.5	13.0	0.5	6.4	1
All attributes	33	1.4	12.5	0.5	5.5	0.5

[1]Weibull's distribution was used with a probability of finding a failed product of F(X) = 50%.

[2]The lower confidence limit was 37 − 15 and the upper confidence limit was 37 + 26.

regression of log(x) versus log[H(x)] (see Equation [1]) is shown in Figure 2 for the flavor data at 6°C with the corresponding Working-Hotelling confidence intervals (12). Combining Equations [1] and [2], the points and curve of Figure 3 were obtained from the points and straight line of Figure 2, respectively. Figure 3 is of practical importance: an estimation can be obtained of the impact on shelf-life when the F(x) criteria is changed. If, for example, we can only tolerate a 10% probability of product failure, the shelf-life would be reduced to 27 d instead of 33 d calculated for F(x) = 50%. Conversely, increasing shelf-life to 40 d would result in a 95% chance of product failure.

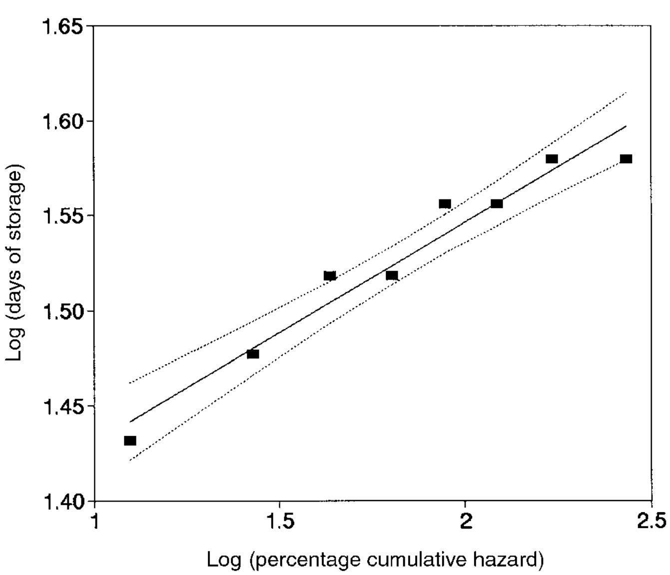

Figure 2. Logarithm of days of storage versus logarithm of cumulative hazard with 95% confidence intervals for flavor of Ricotta cheese stored at 6°C.

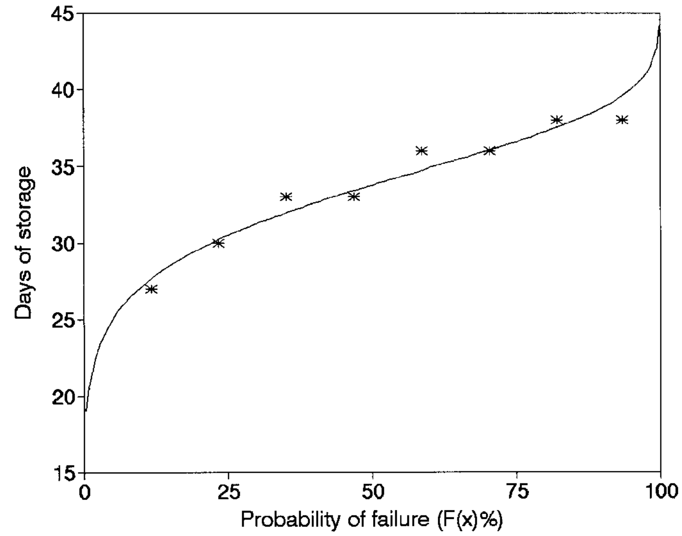

Figure 3. Days of storage as a function of the probability of finding a failed sample for Ricotta cheese stored at 6°C.

Labuza (16) reported that plotting log (days of shelf-life) versus temperature gives a straight line for most food products, which was true for our data. From the least squares regression of log (days of shelf-life) versus temperature, Q_{10} was calculated.

$$Q_{10} = \frac{\text{shelf-life at T°C}}{\text{shelf-life at (T + 10°C)}} = \frac{\text{shelf-life at 6°C}}{\text{shelf-life at 16°C}} = 2.52.$$

Activation energy and Q_{10} can be related by

$$Q_{10} = \frac{\text{reaction rate at (T + 10°C)}}{\text{reaction rate at T°C}} = \frac{k_{T+10} \times A^n}{k_T \times A^n}. \quad [3]$$

where: k = reaction rate constant, A = concentration, n = exponent defining reaction order. From the Arrhenius equation activation energy (Ea) is determined by

$$k = k_0 \times e^{-Ea(R \times T)}. \quad [4]$$

where: k_0 = constant, R = gas law constant. Placement of Equation [4] in Equation [3] and rearranging gives:

$$Q_{10} = e^{\frac{Ea}{R}\left[\frac{1}{T + 10} - \frac{1}{T}\right]}$$

from which Ea can be calculated as follows:

$$Ea = \log(Q_{10}) \times T(°K) \times [T(°K) + 10] \times 4.57 \times 10^{-4} = 14.8 \text{ kcal/mol.}$$

Both Q_{10} and Ea values are within the ranges reported for other dairy products (16).

CONCLUSIONS

Spearman's rank correlation test showed significant association between total aerobic mesophile growth and changes in flavor and titrable acidity during storage of Ricotta cheese at different temperatures.

Weibull's distribution proved to be an adequate model to predict shelf-life of Ricotta cheese based on goodness of fit criteria and acceptable confidence limits. A curve was drawn, which allowed estimation of probability of product failure for different storage times.

Shelf-life at different temperatures allowed calculation of Q_{10} and the corresponding activation energy. These values were in the range published for other dairy products.

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