Estimation of the Shelf-Life of Halloumi Cheese Using Survival Analysis

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Abstract

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Halloumi cheese blocks, packaged in vacuum polyamide/polyethylene laminate bags, were stored at 5, 15, and 25°C. The changes in total bacterial count, lactic acid bacteria, total anaerobic bacteria, yeasts and molds, pH, and titratable acidity were monitored during the storage. The appearance of the packaged Halloumi cheese exhibited significant correlations with the counts of the different microbial populations inhabiting the cheese. The shelf-life of the stored Halloumi cheese was determined using survival analysis and considering consumer rejection as a failure index. The nominal shelf-lives of Halloumi cheese were 79.6, 37.8, and 2.6 days when stored at 5, 15, and 25°C, respectively. The Q_{10} values (shelf-life at T °C/shelf-life at T + 10°C) at 5°C and 15°C were 2.1 and 14.5, respectively. The increase in the counts of different microbial populations during storage highlights the need for adherence to good manufacturing practices and maintenance of low temperatures during the storage and distribution of the packaged Halloumi cheese.

Keywords: brined cheese; microbiology; sensory; Q_{10}

Halloumi cheese is the traditional cheese of Cyprus. The consumption of Halloumi has become widespread and the cheese is currently manufactured on an industrial scale in the North East Mediterranean and the Balkans (Bintsis & Papademas 2002). In addition, Halloumi is exported to the European Union and North America to meet the demands of consumers for this cheese (Papademas 2006).

Halloumi is produced from bovine, caprine, and ovine milks or from a mixture of different types of milk and is consumed raw, grilled, or fried. Halloumi cheese is yellow when produced from bovine milk and white when made from ovine or caprine milks (Papademas & Robinson 1998). The quality and shelf-life of Halloumi are affected by several factors including the quality of milk and the hygienic practices during processing (Bintsis & Papademas 2002).

In Cyprus, fresh Halloumi is made from pasteurised milk. After pasteurisation and cooling, the milk is coagulated by rennet for 30 min and the resulting coagulum is cut to 1 cm^3 cubes which are then allowed to rest for 10 min and pressed into blocks at 300 kPa. The pressed blocks are boiled in whey for 1 h, drained, cooled, and stored in whey brines (12% NaCl). Mature Halloumi cheese is produced by storing the fresh cheese in the whey brine (12% NaCl) for 40 days at 15–20°C (Papademas & Robinson 1998; Papademas 2006).

In Lebanon and other Middle Eastern countries, Halloumi is made in a manner similar to that described for fresh Halloumi with slight modifications. The cheese blocks are boiled for 30 min and then stored overnight, for 16–18 h, in 12–14% brine. The next day, the cheese blocks are packed in vacuum plastic bags and displayed under refrigeration (5–8°C). The shelf-life of Halloumi cheese as stated by different regional producers ranges between 4 and 6 months under refrigeration.

Although Halloumi cheese is boiled in whey, several studies have shown the presence of different types of microorganisms due to the low quality of milk (Milci *et al*. 2005) or due to the lack of proper hygiene during processing (Dib *et al*. 2008). Thermoduric microorganisms survive the boiling process and lactic acid bacteria have also been reported to be present in fresh and mature Halloumi cheese (Poullet *et al.* 1993). The sensory properties of Halloumi cheese are also affected by *Lactobacillus cypricasei* which thrives in the whey brines and possesses a wide range of enzymatic activities (Papademas & Robinson 2002).

Estimating the shelf-life of foods is essential to protect the consumer health and for successful marketing (Schmidt & Bouma 1992). The determination of the shelf-life of foods entails fitting the failure times based on sensory, chemical, and microbiological cut-off points to different statistical distributions and calculating the expected time-to-failure at different probability levels (Gacula *et al.* 2009). This approach has been utilised in the determination of the shelf-life of foods using either trained panelists to determine the limits of sensory acceptability (Hough *et al.* 1999; Al Kadamany *et al.* 2002; Guerra *et al*. 2008) or ordinary consumers who are asked to indicate their willingness/refusal to buy/consume the product (Ares *et al.* 2008; Cruz *et al.* 2010). The latter technique, employing regular consumers of the products and survival analysis statistics, is considered as being more reflective of the interaction between the consumers and food products and is increasingly used in the determination of the shelf-life of foods (Hough 2010).

The end of shelf-life of foods is shaped by the biochemical, chemical, microbiological, and physical changes that take place in the food matrix after the production and during the distribution and display. Some of the biochemical, chemical, and microbiological changes that take place during bulk storage of Halloumi cheese in the whey have been reported (Ayyash & Shah 2010). However, and as noted earlier, Halloumi is increasingly being packed in plastic bags in brine after overnight storage and displayed in retail outlets. The microbiological and physicochemical changes which take place in packaged Halloumi and their relationships to the shelf-life of the product have not been investigated. The objectives of this study were to (a) monitor the changes in the selected microbiological and physicochemical parameters, and (b) determine the shelf-life of packaged Halloumi cheese using survival analysis.

MATERIAL AND METHODS

Halloumi cheese-making. Cow's milk (300 kg) was heated at 68°C for 30 min, cooled to 38°C and treated with $CaCl_2$ at 0.20%. Rennet (Chy-Max, minimum strength 2080 International Milk Clotting Units (IMCU)/g; Chr Hansen, Høsholm, Denmark) was added at the rate of 1 g/100 l of milk and the milk was stirred. After resting for 50 min, the resulting coagulum was cut into grains of ~1 cm^3 which were then transferred into square stainless steel molds lined with cheesecloth. The curd was pressed at 400 KPa for 1 h and the pressed curd was then cut into rectangular blocks (15 × 10 × 3 cm). The cheese whey was deproteinised by heating at 95°C for 10 min in the presence of 0.5% citric acid. The pressed curd blocks were heated in the deproteinised whey at 95°C until they floated to the surface (~ 30–35 min). The plasticised cheese blocks were removed from the whey, folded, and allowed to cool on stainless steel surfaces. The folded Halloumi blocks were placed in plastic containers, covered with 12% NaCl preheated brine (100°C, 10 min) and kept overnight (~ 16–18 h) at 4 ± 1°C. The next day, the Halloumi cheese blocks were packaged under vacuum in vacuum bags made of 90 µm polyamide/polyethylene laminate (oxygen transmission rate, 21 cc/m^2 × 24 h × atm; Vacuum Bags s.a.r.l., Roumieh, Lebanon) and brought to the laboratory in a refrigerated truck, randomised, and stored at 5, 15 or 25 ± 1°C.

Storage temperatures and sampling times. The cheeses stored at 5°C were sampled on days 30, 44, 58, 72, 86, 100, 114, 128, 142, 156, and 170; those stored at 15°C were sampled on days 18, 24, 30, 36, 42, 48, and 54, while those stored at 25°C were sampled on days 4, 8, 12, 16, and 20. At each

time of sampling, a randomly chosen sample was removed from the incubator or refrigerator, placed at room temperature for 10 min before it was presented to the consumers. Another randomly chosen sample was subjected to microbiological and physicochemical testing.

Survival analysis. For the determination of the shelf-life of foods by survival analysis, samples of foods are stored for different periods of time and usually presented to the same group of consumers in one session. The consumers evaluate all samples, under the same setting, and indicate their willingness/refusal to consume the products if these were to be offered to them under real life situations (Hough 2010). Under these conditions, the samples are removed from storage and kept at low temperatures (chilling or freezing) to minimise any further changes. In the present work, the chilling of the samples at temperatures below 5°C (0°C < temperature < 5°C) is not expected to arrest the biochemical and microbiological changes in the samples stored at 5°C; keeping the samples at freezing temperatures would cause marked changes in the texture of the cheese. These limitations coupled with the impracticality of assembling the same panelists at the experimentally-chosen storage periods and temperatures precluded the presentation of all the samples to the same panelists. Under these conditions, current-status survival analysis is carried out whereby different panels are assembled at different storage periods and each panelist assesses one sample at each storage period and temperature (Hough 2010).

In the shelf-life studies, the food samples are assessed sensorially by taste and/or smell after storage for different periods of time. This approach could not be followed in the current study due to the possible exposure of the panelists to health hazards residing especially in that the samples had a relatively-high pH (~ 6.3) and were expected to contain high microbial loads at the later stages of storage. Instead, the panelists were asked to look at the samples, varying by the storage period and temperature, and to indicate their willingness/refusal to buy and consume the samples. This approach had been utilised previously in the determination of the shelf-life (Ares *et al.* 2008; Torrieri *et al.* 2008), given the potential health hazards that might result from consuming cheese samples stored for relatively long periods of time. Accordingly, at each sampling time, a randomly chosen cheese sample was presented to 40 consumers (balanced for males and females, age 18–60 years) who were asked to look at the sample and to indicate whether they would purchase the sample. A total of 920 consumers of Halloumi cheese participated in the evaluation of the samples stored for the indicated periods of time at the different storage temperatures (11 panels for the samples stored at 5°C, 7 panels for the samples stored at 15°C, and 5 panels for those stored at 25°C).

Microbiological and physicochemical determinations. At each sampling time, a cheese sample was chosen at random and subjected to microbiological and physicochemical analysis.

The counts of total bacteria, total anaerobic bacteria, lactic acid bacteria, and yeasts and molds were determined according to standard procedures (Wehr & Frank 2004). Twenty grams of cheese were blended with 180 g of sterile distilled water in a stomacher (Daigger, Vernon Hills, USA) to obtain a homogenous mixture and further dilutions were done when necessary. Total bacterial counts (TBC) and total anaerobic bacteria (TAB) were enumerated by pour plate technique using plate count agar (Bio-Rad Laboratories Inc, Cellini, Italy). The plates for TBC were incubated at 37°C for 72 h and those for TAB were incubated at 30°C for 72 h under anaerobic conditions. Lactic acid bacteria (LAB) were also enumerated by the pour plate technique using MRS Agar (Bio-Rad Laboratories Inc, Cellini, Italy), the plates having been incubated at 30°C for 72 hours. Yeasts and molds (Y&M) were enumerated using yeast glucose chloramphenicol media (YGC agar; Bio-Rad Laboratories Inc, Cellini, Italy); the plates were incubated, under aerobic conditions, at 25°C for 5 days. All microbiological analyses were performed in duplicate.

pH values were determined using a digital pH meter (Model 51950; Hach, Loveland, USA) and by inserting the electrode into the cheese sample (Papaionnou *et al.* 2007). The titratable acidity in the cheese samples was determined according to AOAC (2007; Method 920.124) by titrating an aliquot of the filtrate obtained by filtering a suspension of the cheese (10 g in 105 ml distilled water) with 0.1M NaOH using phenolphthalein as indicator; the titratable acidity was expressed in g lactic acid/100 g cheese. All chemical analyses were performed in triplicate.

Statistical analyses. The association between the different microbiological and physicochemical parameters and consumers' acceptability/rejection

of Halloumi cheese was analysed by the Spearman's correlation test (Gacula *et al.* 2009) using SPSS software (SPSS 2009).

The current-status survival analysis methodology, where different panels are used in different sessions and each subject assesses one sample in a session, yields censored data. If a subject accepts the product stored for the indicated period, his/her verdict is right-censored and if he/she rejects the product his/her assessment is left-censored (Hough 2010). The obtained censored data were fitted to the statistical distributions that are most often used in the shelf life studies, specifically the Weibull, lognormal, loglogistic, and exponential (Cruz *et al.* 2010; Jacobo-Velázquez *et al.* 2010). The parameters of the different distributions and their log likelihoods were determined by maximum likelihood estimation using the R statistical package (2010).

RESULTS AND DISCUSSION

The total bacterial count, total anaerobic bacteria, lactic acid bacteria, and yeasts and molds counts, as well as the changes in pH and titratable acidity levels at the different storage temperatures are presented in Figure 1.

TBC increased steeply in cheeses stored at 25°C and reached 3.95 $\log_{10}$ CFU/g at 20 day while at 15°C it exhibited a sharp increase in the early stages of storage and increased slowly thereafter reaching 4.71 $\log_{10}$ CFU/g at the end of storage at 54 day. At 5°C, TBC reached 4 $\log_{10}$ CFU/g at 114 day of storage and showed a small change afterwards in the investigated storage period of 170 days. In general, TBC increased with an increase in storage temperature in line with previous reports on TBC of Halloumi cheese stored in brine (Papademas & Robinson 1998).

Lactic acid bacteria counts (LAB) increased gradually as the storage progressed. The LAB count of 0.9 $\log_{10}$ CFU/g in freshly-packed Halloumi reached 3.38, 3.64, and 3.43 $\log_{10}$ CFU/g at 25°C/20 days, 15°C/54 days and 5°C/170 days, respectively. The LAB have been reported to survive the heat treatment step during processing and to multiply during bulk storage of Halloumi cheese in brine (Ayyash & Shah 2010).

The growth of yeasts and molds was affected more by the storage temperature than the storage period. Yeasts and molds counts reached 2.62, 2.55, and 3.17 $\log_{10}$ CFU/g after storage for 170, 54, and 20 days at 5, 15, and 25°C, respectively. Yeasts and molds are believed to originate from the environment of the dairy and the whey brines in which the cheeses are immersed prior to packaging (Papademas 2006).

Total anaerobes increased during the early stages of storage and showed a small change as the storage progressed. Total anaerobic counts reached 3.08, 2.84, and 3.06 $\log_{10}$ CFU/g at the end of storage at 5, 15, and 25°C, respectively. A number of species of *Bacillus* and *Clostridium* have been reported to thrive under the low pH and high salt content of white-brined cheeses (Bintsis & Papademas 2002).

The titratable acidity increased and reached 0.20 g/100 g cheese in samples stored at 25°C, 0.17 g/100 g cheese in samples stored at 15°C, and 0.36 g/100 g cheese in samples stored at 5°C after 20, 54, and 170 days, respectively. The contents of organic acids have been reported to increase during storage of Halloumi cheese presumably due to the catabolism of lactose and amino acids by the varied microbial populations established in the product during storage (Kaminarides *et al*. 2007; Papaionnou *et al*. 2007). The increase in acidity was matched by the drop in pH of the

Table 1. Spearman's rank correlation coefficients between microbial counts and pH, acidity and consumer's rejection

	pH			Titratable acidity			Number of failure responses		
	25°C	15°C	5°C	25°C	15°C	5°C	25°C	15°C	5°C
TBC	–0.952**	–0.766**	–0.923**	0.946**	0.731*	0.868**	0.898**	0.850**	0.755*
TAB	–0.790*	–0.747*	–0.410	0.837**	0.711*	0.223	0.759*	0.825*	0.383
Y&M	–0.952**	–0.724*	–0.851**	0.946**	0.626	0.692*	0.898**	0.773*	0.705*
LAB	–0.952**	–0.766**	–0.881**	0.964**	0.731*	0.732**	0.898**	0.850**	0.775**

Titratable acidity – g of lactic acid/100 g cheese; number of failure response – number of consumers who refused to buy the cheese; TBC – total bacterial count; TAB – total anaerobic bacterial count; Y&M – yeasts and molds; LAB – lactic acid bacteria; *$P < 0.05$; **$P < 0.01$

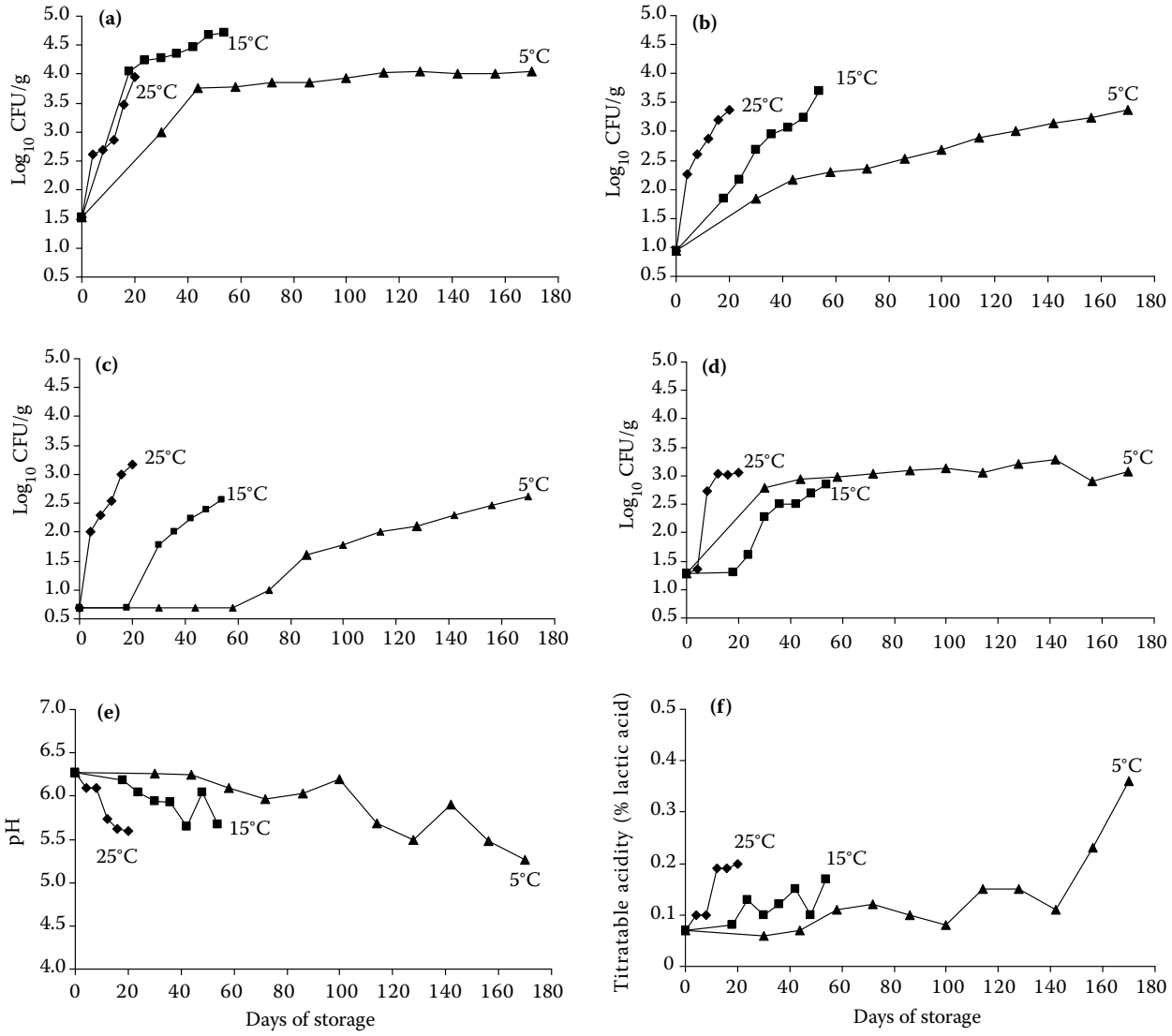

Figure 1. Changes in (a) total bacterial count (TBC), (b) lactic acid baerobic (TA), (e) pH, and (f) titratable acidity (% lactic acid) during storage of packaged Halloumi cheese at 5, 15, and 25°C

Maximum standard deviations of means of the $\log_{10}$ CFU/g were 0.12, 0.47, 0.04 and 0.08 for TBC, LAB, Y&M, and TA respectively; maximum standard deviations of means were 0.05 and 0.03 for pH and titratable acidity, respectively

samples irrespective of the storage temperature. The pH of the fresh samples was 6.27 and reached 5.60 after 20 days at 25°C, 5.67 after 54 days at 15°C, and 5.27 after 170 days at 5°C. The pH of the samples were within the range (4.8–6.2) reported for commercial Halloumi samples (Papademas 2006).

Total anaerobes exhibited significant correlations ($P < 0.01, 0.05$) with pH and titratable acidity in the samples stored at 15°C and 25°C and, apart from a non-significant relation with titratable acidity in the samples stored at 15°C, Y&M counts correlated ($P < 0.01, 0.05$) with pH and titratable acidity in the samples (Table I). The pH and titratable acidity correlated significantly ($P < 0.01, 0.05$) with TBC and LAB counts irrespective of the storage temperature (Table 1), thereby indicating that the changes in cheese acidity during storage are largely modulated by the growth of lactic acid and aerobic bacteria. Moreover, pH correlated significantly ($P < 0.05$) with the product failure (consumers' rejection) for the samples stored at 5°C ($r = -0.702$) and 25°C ($r = -0.850$). Titratable acidity correlated significantly ($P < 0.05$) with consumers' rejection with correlation coefficients of 0.611, 0.802, and 0.831 for the cheese samples stored at 5, 15, and 25°C, respectively. The significant correlations ($P < 0.01, 0.05$) between the product failure (con-

sumers' rejection) and microbiological parameters and the products acidity suggest that the cheese appearance is highly affected by the growth of TBC, yeasts and molds, lactic acid bacteria, and the acids produced through their metabolic activities. The growth of the non starter lactic acid bacteria has been shown to affect the appearance of cheese (Somers *et al.* 2001) and the increase in acidity during storage has been reported to influence the structure and shelf-life of Crescenza cheese (Benedetti *et al.* 2005).

Determination of shelf-life

The cheese samples exhibited different defects as the storage progressed. The participants expressed their unwillingness to buy the samples which had lost their luster, those with dry yellow patches and/or those exhibiting syneresis which became apparent especially at the advanced stages of storage. Among the distributions used to model the data, the Weibull distribution had the lowest log likelihood and was, therefore, considered as the best-fitting model (Hough 2010). The Weibull rejection function $F(t)$ is defined as:

$$F(t) = 1 - \exp\left[-\exp\left(\frac{\ln(t) - \mu}{\sigma}\right)\right]$$

where:

t – failure time and

μ, σ – parameters of the Weibull distribution

Plotting the Weibull distribution at the determined μ and σ yields a curve relating the probability of the product rejection to the storage time. The parameters of the Weibull distributions and the corresponding curves depicting the probability of the product rejection *vs* storage time are presented in Table 2 and Figure 2.

As expected, the time needed to reach the same probability of the product rejection decreased as storage temperatures increased (Figure 2). The nominal shelf-life of food products is usually determined at 50% probability of the product rejection (Gacula *et al.* 2009). Using this criterion, the nominal shelf-life of Halloumi cheese is expected to be 79.6, 37.8, and 2.6 days at 5, 15, and 25°C, respectively (Table 2).

The determination of the shelf-life at different temperatures is very often used to derive the values for Q_{10}, which defines the change in shelf-life of foods at storage temperatures differing by 10°C, and the activation energy (E_a), which gives a measure of the temperature dependence of the sensory quality change upon storage (Hough *et al.* 1999; Al Kadamany *et al.* 2002). These values are of practical significance in predicting the changes in the shelf-life with variations in temperatures during the distribution and storage of foods (Taoukis *et al.* 1997). As noted by different workers (Hough 2010; Torri *et al.* 2010), these parameters provide useful indices for the shelf-life characterisation when the Q_{10} values determined are reasonably similar at different temperatures. The computation of the Q_{10} values for the shelf-life of Halloumi cheese yielded 2.1 and 14.5 at 5°C and 15°C, respectively. The marked differences in the computed Q_{10} values indicate the operation of different failure modes in the temperature range of 5–15°C and at 25°C. These findings further indicate that extrapolation of the data obtained at

Table 2. Values of the Weibull distributions' parameters μ and σ and the estimated shelf lives of Halloumi cheese at the investigated storage temperatures

Storage temperature (°C)	Weibull distribution		Shelf life (days) (95% CI)
	μ (95% CI)	σ (95% CI)	
5	4.82 (4.64–5.00)	1.22 (0.86–1.73)	79.6 (66.5–95.1)
15	4.03 (3.71– 4.34)	1.08 (0.61–1.92)	37.8 (31.3–45.7)
25	1.45 (0.99–1.91)	1.31 (0.83–2.08)	2.6 (1.4–5.1)

CI – confidence interval; shelf life – calculated at 50% probability of rejection by consumers

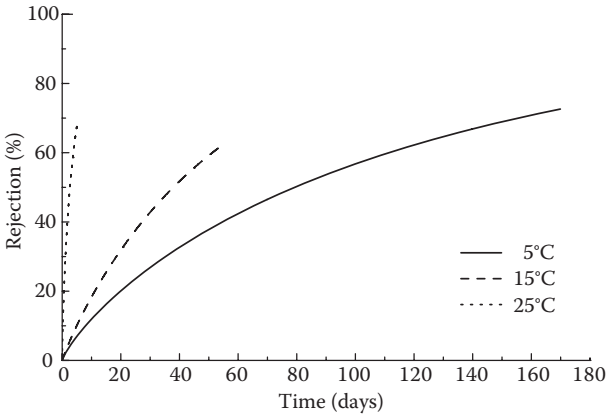

Figure 2. Consumers' rejection to purchase as a function of storage time packaged Halloumi cheese stored at 5, 15, and 25°C

high temperatures to predict the shelf-life at lower temperatures must be approached with caution.

The sensitivity of the shelf-life of packaged Halloumi to temperature and the dramatic shortening of the shelf-life observed at 25°C highlight the need to maintain low chilling temperatures during the distribution and storage. The end of the shelf-life of foods has often been related to the microbiological counts and/or values of physicochemical parameters in different products categories (Calligaris *et al.* 2007; Duyvesteyn *et al.* 2001). At storage temperatures of 5°C, and considering a failure probability of 50%, packaged Halloumi cheese is expected to have TBC of 3.8–4.0 $\log_{10}$ CFU/g, LAB of 2.4–2.6 $\log_{10}$ CFU/g, TAB of 3.0–3.1 $\log_{10}$ CFU/g, yeasts and molds of 0.90–1.5 $\log_{10}$ CFU/g, pH of 6.0–6.2, and titratable acidity of 0.06–0.12 g lactic acid/100 g cheese.

CONCLUSION

Total aerobes, total anaerobes, yeasts and molds, and lactic acid bacteria increased during storage of packaged Halloumi cheese. The increase in the counts of the aforementioned groups of microorganisms led to a drop in the pH and a rise in the titratable acidity of Halloumi. The microbial activities brought about changes in the appearance of the cheese leading to its rejection by the consumers. The life data obtained by survival analysis using regular consumers of Halloumi were adequately described by the Weibull distribution. The nominal shelf-life of Halloumi stored at 5°C was 79.6 days and dropped to 37.8 days at 15°C suggesting the conformity of the quality changes in the product to the Q_{10} value of 2. However, the dramatic drop in the shelf-life of the product stored at 25°C to 2.6 days was caused by detrimental changes in the cheese appearance. The dramatic shortening of Halloumi cheese shelf-life observed at 25°C highlights the need to maintain low temperatures during the product distribution and display. Furthermore, the present data indicate that consumers' acceptance of the product can be increased by lowering the initial microbial load of the products through adherence to good manufacturing practices and institution of effective quality systems.

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