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Research Note

Effects of Flavorings, Storage Conditions, and Storage Time on Survival of *Staphylococcus aureus* in Sürk Cheese

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ABSTRACT

The objectives of this study were to determine the cumulative effects of flavorings (chili pepper, thyme, mint, cumin, nutmeg, allspice, clove, cinnamon, black pepper, salt, and hot red pepper paste), storage conditions, and storage time on the survival of *Staphylococcus aureus* in Sürk cheese and to monitor the associated chemical changes. Sürk cheese, a traditional Turkish cheese, was produced by heating diluted nonfat yogurt and adding flavorings to the resultant acid-heat curd. The cheese was later inoculated with *S. aureus*, shaped conically, and stored aerobically for mold growth and anaerobically in olive oil for 30 days at room temperature. The moisture content of aerobically stored cheese decreased over time and led to increases in total solids, salt, salt-in-moisture, and ash content during ripening ($P < 0.05$). The presence or absence of the flavorings had no significant effect, whereas storage conditions and storage duration decreased the survival of *S. aureus* ($P < 0.05$).

Sürk cheese is a traditional spicy cheese of Turkey most commonly made in the province of Hatay. Sürk cheese is traditionally composed of an acid-heat curd obtained by heating diluted nonfat finished yogurt and different types of flavorings (chili pepper, thyme, mint, cumin, nutmeg, allspice, clove, cinnamon, black pepper, salt, and hot red pepper paste). Sürk cheese is of a conical shape with an approximate diameter of 5 to 7 cm. For ripened consumption, each cheese ball is wrapped in a piece of paper and placed in a jar for approximately 30 days at room temperature to promote mold growth. For fresh consumption, mold growth is prevented by either keeping the cheese balls in olive oil or covering the cheese ball surface with olive oil before wrapping with stretch film.

Information is lacking about the origin, traditional production methods, and composition of Sürk cheese *(6, 15)*. However, antibacterial effects of essentials oils of some spices used in Sürk cheese on common food pathogens have been recently studied extensively *(5, 9, 10, 16, 25)*, and the components responsible for possible inhibition mechanisms have been revealed *(1, 8, 18, 28)*. Most studies were performed with individual essential oils in vitro. However, direct use of essential oils instead of flavorings as an antimicrobial agent in real food systems is not a common application. Few attempts have been made to directly use spices alone *(29, 31)* or in combination with other food preservatives as antimicrobial agents *(7, 32)*.

Sürk cheese is commonly made in the home with bare hands and, hence, is more prone to contamination. What makes Sürk cheese unique among other cheeses is the use of the flavorings that may act as preservatives. These properties of Sürk cheese provide a good medium to assess the fate of foodborne pathogens. Objectives of the study were therefore (i) to determine the cumulative effects of the flavorings in Sürk cheese, aerobic and anaerobic storage conditions, and storage duration (30 days at room temperature) on the survival of *Staphylococcus aureus* and (ii) to monitor associated chemical changes.

MATERIALS AND METHODS

Raw milk. Twenty-five liters of raw cow's milk was obtained from the Research and Training Farm of Mustafa Kemal University (Antakya, Turkey). Morning milk was cooled to 4°C immediately after machine milking and brought in a stainless steel container for processing within 45 min after cooling.

Bacterial cultures. Lyophilized commercial yogurt culture (DRI-VAC, YC-360) was purchased from Peyma-Hansen (Istanbul, Turkey). The whole sachet (6 to 7 g) was added to 500 ml of sterilized skim milk prepared from skim milk powder (10% total solids) and mixed for approximately 30 min in a water bath at 43 ± 1°C to dissolve completely before its addition to bulk milk (20 liters) at the level of 0.2% (vol/vol). *S. aureus* (ATCC 29213) was obtained from the culture collections of the Department of Health of Refik Saydam Hygiene Center, Research Department of Contagious Diseases (Ankara, Turkey). *Staphylococcus* culture was activated twice in tryptone soy broth (Difco, Becton Dickinson, Sparks, Md.) at 37°C overnight before use.

Flavorings. Mint, cumin, nutmeg, allspice, cinnamon, clove, and black pepper powder were obtained from Bagdat Baharatları Ltd. Co. (Ankara, Turkey). According to the product sheet, the flavorings were already sterilized by gamma irradiation. Thyme, red pepper, salt, and hot red pepper paste were obtained from a local market. As described in the ''Microbial Analyses'' section, all the flavorings bought from the local market were tested for the

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presence of *S. aureus* before use, and only those without *S. aureus* were used in cheese making (data not shown).

Olive oil. Riviera-type olive oil was purchased from the local market (Antakya, Turkey).

Cheese making. Twenty-five liters of raw cow's milk was heated to 50 to 60°C and the fat content removed with an electrically driven laboratory scale separator (Sübitaş, Istanbul, Turkey). The resulting skim milk (20 liters) was heated (90°C for 5 min) in stainless steel containers (Saruhan Mak. San., Konya, Turkey), placed in a boiling water bath, and cooled (43 ± 1°C) with running tap water. The skim milk was inoculated with previously prepared yogurt cultures (0.2%, vol/vol) and incubated (43 ± 1°C for 5 to 5.5 h) until yogurt gel formed. The gel was broken and heated (90°C for 10 min) to produce an acid-heat curd, which was drained with the cloth-bag method and pressed overnight at room temperature. The resultant acid-heat curd was divided into equal halves. Flavorings were added to one half (chili pepper, 0.85%; thyme, 0.50%; mint, 0.15%; cumin, 0.50%; nutmeg, 0.10%; allspice, 0.10%; clove, 0.10%; cinnamon, 0.10%; black pepper, 0.10%; hot pepper paste, 6.0%; and salt, 1.50%), which was then kneaded and used to make cheese balls weighing 50 g (referred to as S cheese). The other half was subjected to the same treatment (without the addition of the flavorings) and used as the control group (referred to as C cheese). All the cheese balls were then inoculated with 100 μl of enriched culture of *S. aureus* (ATCC 29213) corresponding to approximately 10^6 CFU/g. Cheese balls were cut in half, and *S. aureus* culture was spread with a pipet onto the cut surface of one half of the ball. The halves were put back together, kneaded slightly to facilitate the distribution of the bacteria, and given a conical shape. The balls were stored in a room without direct sunlight at room temperature for a day for drying. Later, half of the S and C cheese balls were subjected to anaerobic conditions by being kept in jars with olive oil (referred to as S1 and C1 cheeses). The other half of the balls were subjected to aerobic conditions by wrapping them with pieces of water-permeable filter paper (Whatman no. 1) and placing them in jars (referred to as S2 and C2 cheeses). The cheese balls were stored for 30 days at room temperature. During the first 10 days, the jars were kept open to facilitate drying as is done traditionally. After 10 days, the jars were closed tightly to prevent escape of moisture. The cheese production was performed six times, three for microbiological analyses and three for chemical analyses.

Compositional analyses. Moisture and ash contents of the samples were determined gravimetrically *(4)*. Total solids content was calculated by subtracting the moisture content from 100. The Mohr method was used to determine the salt content of the cheese samples as described by Richardson *(24)*. Salt-in-moisture content was calculated from the salt and moisture content. pH measurements were made according to a previously described method *(3)*. For determination of total nitrogen and soluble nitrogen, the cheese samples were prepared according to Gripon et al. *(14)* and analyzed according to a previously described method *(2)*, but on a smaller scale. Water activity (a_w) was calculated from ash and soluble nitrogen contents *(11)*. Chemical analyses were conducted on storage days 0, 1, 3, 5, 10, 15, 20, and 30.

Microbial analyses. Enumeration of *S. aureus* in the flavorings and cheeses, total mold and yeast (TMY), and total mesophilic aerobic bacteria (TMAB) in cheese were performed according to Harrigan and McCance *(17)*, using Baird Parker agar with egg yolk tellurite, potato dextrose agar acidified with 10% (wt/vol) tartaric acid, and plate count agar, respectively. For microbiological analyses, a whole cheese ball (approximately 50 g) was mixed with 450 ml of 0.1% peptone water (wt/vol) by a stomacher (Bagmixer 400, Interscience, St. Nom La Bretéche, France), and appropriate dilutions were surface plated. Typical colonies of *S. aureus*, black shiny colonies of 1.5 to 2.0 mm in diameter surrounded by an opaque zone, were counted. *S. aureus* counts were made on storage days 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, and 30 and TMY and TMAB counts on storage days 0, 1, 3, 5, 10, 15, 20, and 30. Counts were taken in duplicate with the use of potato dextrose agar plates at 22 ± 2°C for 5 days, plate count agar plates at 35 ± 1°C for 48 h, and Baird Parker agar with egg yolk tellurite plates at 35 ± 1°C for 48 h. The agars, egg yolk tellurite, and tartaric acids were obtained from Merck KGaA (Darmstadt, Germany).

Statistical analysis. Data were analyzed using Minitab software version 12.1 (Minitab Inc., State College, Pa.). Three-way analysis of variance and the Duncan's multiple comparison test were used to determine significant differences in response means at the significance level of 0.05. Log transformations were performed on microbial data.

RESULTS AND DISCUSSION

Chemical properties of Sürk cheese. Chemical parameters of Sürk cheese changed during the storage, depending on the addition of the flavorings and/or storage conditions (Table 1). In general, total solids, total nitrogen, soluble nitrogen, salt, salt-in-moisture, and ash values of S2 and C2 cheeses increased significantly as the storage time progressed ($P < 0.05$), whereas those of S1 and C1 cheeses after being placed in olive oil did not change ($P > 0.05$). The loss of moisture may account for the increases in total solids, total nitrogen, salt, salt-in-moisture, and ash content. However, a significant increase in soluble nitrogen from day 5 to day 10 may be attributed to the high proteolytic activity of TMY, which contaminated the cheese balls during their processing and storage *(22)*. The significant increase in soluble nitrogen content coincided with the growth period of TMY, which peaked on day 10 (Fig. 2). Similar results were reported for surface mold ripened cheeses *(22, 27)*.

Changes in soluble nitrogen and ash content were negatively correlated with a_w (Table 1). Early in storage, addition of the flavorings resulted in significantly lower a_w values for S1 and S2 cheeses than for C1 and C2 cheeses ($P < 0.05$). As storage time increased, a_w values in S2 and C2 cheeses decreased progressively ($P < 0.05$), whereas those of S1 and C1 remained almost constant ($P > 0.05$).

An interaction effect of the flavorings, the storage condition, and the storage duration on pH was observed (Table 1) ($P < 0.05$). Addition of the flavorings resulted in high pH values during the early days of ripening, thus suggesting the neutralizing or dilution effect of the flavorings ($P < 0.05$). However, pH began to increase considerably from 4.0 and 4.1 on day 5 to 5.3 and 5.6 on days 10 and 15 in S2 and C2 cheeses, respectively. The increase in pH closely followed the growth pattern of TMY, which suggests that the acid was neutralized by TMY through lactate utilization or ammonia production *(22, 27)*.

Survival of *S. aureus*. Addition of the flavorings had no significant effect on the growth of *S. aureus* (Fig. 1) ($P > 0.05$). The number of *S. aureus* in S1 and C1 cheeses

TABLE 1. *Changes in the chemical composition of Sürk (S) and control (C) cheeses stored under anaerobic (S1, C1) and aerobic (S2, C2) conditions for 30 days at room temperature*[a]

Composition	Cheese	Value on day: 0	1	3	5	10	15	20	30
Total solids (%)	S1	29.4 ± 2.43 J	32.9 ± 2.26 GHI	33.9 ± 1.60 GH	34.3 ± 2.05 G	33.5 ± 1.36 GHI	33.6 ± 1.14 GHI	34.0 ± 1.30 GH	34.3 ± 1.58 GH
	C1	27.4 ± 0.38 K	31.6 ± 1.40 I	32.3 ± 0.92 HI	32.8 ± 0.56 GHI	33.9 ± 0.30 GH	33.1 ± 0.26 GHI	33.7 ± 0.31 GH	34.0 ± 0.22 GH
	S2	29.4 ± 2.43 J	32.9 ± 2.26 GHI	37.1 ± 1.88 EF	38.5 ± 2.07 DE	40.9 ± 1.58 BC	41.9 ± 3.05 BC	42.7 ± 0.21 AB	44.3 ± 1.22 A
	C2	27.4 ± 0.38 K	31.6 ± 1.40 I	34.4 ± 1.86 G	36.4 ± 2.22 F	40.2 ± 0.22 CD	42.2 ± 3.53 B	42.7 ± 2.17 AB	44.4 ± 2.25 A
Total nitrogen (%)	S1	3.08 ± 0.27 I	3.74 ± 0.16 GHI	4.44 ± 0.52 EFG	4.38 ± 0.21 EFG	4.45 ± 0.18 DEFG	4.26 ± 0.32 FGH	4.32 ± 0.20 FG	4.40 ± 0.18 EFG
	C1	3.44 ± 0.17 I	4.50 ± 0.37 DEF	4.84 ± 0.56 CDEF	4.41 ± 1.23 EFG	5.10 ± 0.25 CDE	4.64 ± 0.73 DEF	4.38 ± 0.08 EFG	4.71 ± 0.21 DEF
	S2	3.43 ± 0.35 I	3.74 ± 0.16 GHI	4.20 ± 0.20 FGH	4.56 ± 0.71 DEF	5.93 ± 0.07 A	6.04 ± 0.17 A	5.86 ± 0.57 AB	5.50 ± 0.38 ABC
	C2	3.59 ± 0.38 HI	4.50 ± 0.37 DEF	4.81 ± 0.30 CDEF	5.17 ± 0.52 BCD	5.84 ± 0.60 AB	6.07 ± 0.36 A	5.81 ± 0.08 AB	5.47 ± 0.12 ABC
Soluble nitrogen (%)	S1	0.23 ± 0.05 I	0.27 ± 0.02 I	0.35 ± 0.05 HI	0.53 ± 0.02 GH	1.04 ± 0.00 E	0.70 ± 0.02 FG	0.73 ± 0.09 FG	0.98 ± 0.64 E
	C1	0.23 ± 0.02 I	0.21 ± 0.02 I	0.19 ± 0.00 I	0.13 ± 0.02 I	0.55 ± 0.04 GH	0.29 ± 0.03 I	0.71 ± 0.07 FG	0.64 ± 0.07 FG
	S2	0.28 ± 0.05 I	0.27 ± 0.02 I	0.71 ± 0.05 FG	0.75 ± 0.10 FG	3.49 ± 0.16 BC	3.26 ± 0.13 D	3.56 ± 0.11 B	4.11 ± 0.50 A
	C2	0.23 ± 0.02 I	0.30 ± 0.17 I	0.79 ± 0.07 F	0.62 ± 0.20 FG	3.48 ± 0.33 BC	3.30 ± 0.23 CD	3.38 ± 0.17 BCD	3.57 ± 0.17 B
Salt (%)	S1	2.0 ± 0.08 E	2.2 ± 0.21 DE	2.1 ± 0.08 DE	2.2 ± 0.05 CDE	2.1 ± 0.06 CDE	2.2 ± 0.14 CDE	2.3 ± 0.18 CD	2.3 ± 0.16 CD
	C1	0.2 ± 0.01 I	0.2 ± 0.03 I	0.1 ± 0.04 I	0.2 ± 0.03 I	0.3 ± 0.02 I	0.2 ± 0.03 I	0.3 ± 0.03 I	0.3 ± 0.05 I
	S2	2.0 ± 0.08 E	2.2 ± 0.21 DE	2.1 ± 0.04 DE	2.4 ± 0.05 C	3.3 ± 0.25 B	3.5 ± 0.46 B	3.5 ± 0.32 B	3.8 ± 0.08 A
	C2	0.2 ± 0.01 I	0.2 ± 0.03 I	0.1 ± 0.01 I	0.3 ± 0.08 I	0.6 ± 0.01 H	1.1 ± 0.06 G	1.2 ± 0.08 G	1.5 ± 0.13 F
Ash (%)	S1	2.8 ± 0.01 EF	2.8 ± 0.13 EF	2.7 ± 0.04 E	2.8 ± 0.06 EF	2.8 ± 0.02 EF	2.7 ± 0.11 EF	2.8 ± 0.04 EF	2.9 ± 0.14 EF
	C1	1.3 ± 0.32 IJK	1.3 ± 0.09 IJKL	1.1 ± 0.14 JKLM	1.0 ± 0.02 M	1.0 ± 0.00 M	1.1 ± 0.09 JKLM	1.0 ± 0.01 LM	1.0 ± 0.03 LM
	S2	2.8 ± 0.01 EF	2.8 ± 0.11 EF	3.3 ± 0.03 D	3.6 ± 0.06 C	3.7 ± 0.13 BC	3.9 ± 0.06 AB	3.9 ± 0.08 AB	4.0 ± 0.14 A
	C2	1.3 ± 0.32 IJK	1.3 ± 0.08 HIJ	1.0 ± 0.07 KLM	1.0 ± 0.05 M	1.1 ± 0.08 KLM	1.4 ± 0.31 HI	1.5 ± 0.26 H	1.8 ± 0.14 G
Salt-in-moisture	S1	2.8 ± 0.16GH	3.1 ± 0.22 EFG	3.2 ± 0.05 EFG	3.3 ± 0.13 E	3.3 ± 0.10 E	3.3 ± 0.15 E	3.5 ± 0.21 E	3.5 ± 0.17 E
	C1	0.3 ± 0.02 K	0.3 ± 0.03 K	0.3 ± 0.03 K	0.3 ± 0.04 K	0.4 ± 0.03 K	0.4 ± 0.01 K	0.4 ± 0.04 K	0.4 ± 0.07 K
	S2	2.8 ± 0.16 FGH	3.2 ± 0.32 EF	3.4 ± 0.05 E	4.0 ± 0.06 D	5.6 ± 0.30 C	6.0 ± 0.87 BC	6.2 ± 0.55 B	6.8 ± 0.18 A
	C2	0.3 ± 0.02 K	0.3 ± 0.03 K	0.2 ± 0.02 K	0.4 ± 0.15 K	1.0 ± 0.02 J	1.9 ± 0.11 I	2.0 ± 0.11 I	2.6 ± 0.17 H
a_w	S1	0.986 ± 0.0003 E	0.986 ± 0.0005 E	0.986 ± 0.0002 E	0.984 ± 0.0003 EF	0.981 ± 0.0001 GH	0.983 ± 0.0007 F	0.983 ± 0.0007 FG	0.981 ± 0.0021 H
	C1	0.994 ± 0.0016 BC	0.994 ± 0.0004 ABC	0.995 ± 0.0007 AB	0.996 ± 0.0001 A	0.993 ± 0.0002 BCD	0.994 ± 0.0003 ABC	0.992 ± 0.0004 CD	0.992 ± 0.0004 CD
	S2	0.985 ± 0.0003 E	0.985 ± 0.0005 E	0.981 ± 0.0003 H	0.979 ± 0.0003 I	0.963 ± 0.0006 MN	0.963 ± 0.0008 M	0.961 ± 0.0007 N	0.958 ± 0.0007 O
	C2	0.994 ± 0.0016 BC	0.993 ± 0.0005 BCD	0.992 ± 0.0001 D	0.993 ± 0.0011 BCD	0.976 ± 0.0022 J	0.976 ± 0.0019 JK	0.975 ± 0.0020 K	0.972 ± 0.0016 L
pH	S1	4.08 ± 0.05 EFG	4.11 ± 0.09 EF	4.11 ± 0.04 EF	4.11 ± 0.06 EF	4.06 ± 0.04 EFGH	4.05 ± 0.06 EFGH	4.17 ± 0.01 E	4.17 ± 0.02 E
	C1	3.87 ± 0.07 HIJ	3.87 ± 0.12 HIJ	3.87 ± 0.04 HIJ	3.87 ± 0.05 HIJ	3.83 ± 0.01 IJ	3.79 ± 0.10 J	3.93 ± 0.03 FGHIJ	3.97 ± 0.01 FGHIJ
	S2	4.08 ± 0.05 EFG	4.11 ± 0.09 EF	4.13 ± 0.04 EF	4.00 ± 0.07 EFGHI	4.68 ± 0.02 D	5.29 ± 0.13 BC	5.37 ± 0.02 B	5.37 ± 0.00 B
	C2	3.87 ± 0.07 HIJ	3.87 ± 0.12 HIJ	3.90 ± 0.04 GHIJ	4.10 ± 0.31 EFG	5.26 ± 0.48 BC	5.60 ± 0.17 A	5.20 ± 0.15 BC	5.16 ± 0.08 C

[a] Data are presented as mean ± standard deviation. Means with different letters in the same row and column for each compound are significantly different ($P < 0.05$).

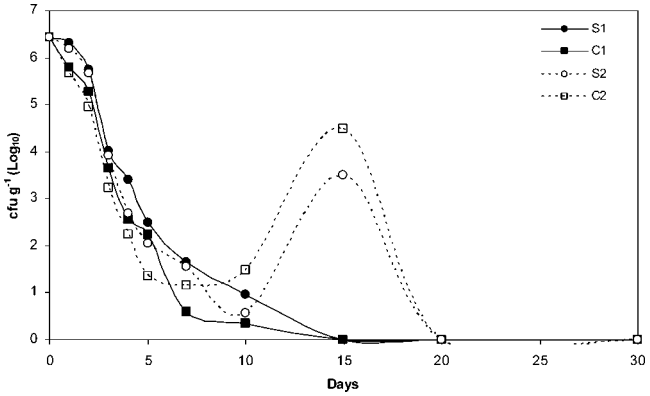

FIGURE 1. *Changes in the number of* Staphylococcus aureus *in Sürk (S1, S2) and control (C1, C2) cheeses stored aerobically (○, □) or anaerobically (●, ■) at room temperature.*

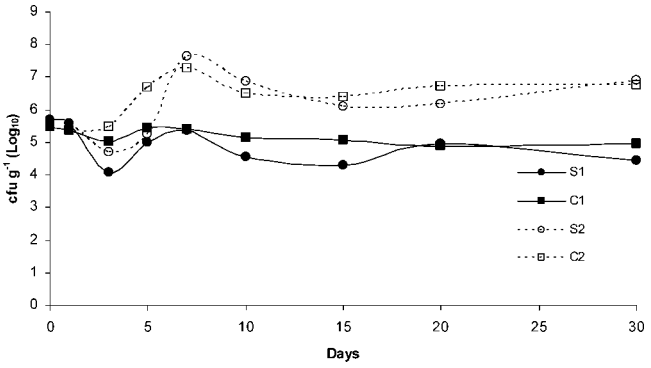

FIGURE 3. *Changes in the number of total mesophilic aerobic bacteria in Sürk (S1, S2) and control (C1, C2) cheeses stored aerobically (○, □) or anaerobically (●, ■) for 30 days at room temperature.*

declined increasingly during ripening ($P < 0.01$), and no colonies were detected on day 15 and later. As could be expected, low pH (approximately 4.0 during storage) prevented the survival of *S. aureus* in S1 and C1 cheeses placed in olive oil *(26)*.

The behaviors of *S. aureus* in S2 and C2 cheeses appeared to be similar to those of S1 and C1 cheeses up to day 7. At this point, the number of *S. aureus* in S2 and C2 cheeses began to increase by approximately 2 log and peaked on day 15. This suggests some changes in the medium favorable for the survival of *S. aureus.* The increase in pH from approximately 4.0 to above 5.0 from day 5 to day 10 was a result of mold growth *(22, 27)*. From day 15 to day 20, a sharp decline in *S. aureus* count was observed, and *S. aureus* could not be counted in the cheese in the remaining days of ripening. Since none of the chemical composition determined accounted for these observations, the decline in *S. aureus* may be attributed to diffusion of the antibiotic-like metabolites produced by molds *(20, 23)* and/or contaminant flora *(12, 21)* or peptides from the degradation of casein *(19)*. The survival pattern of the bacteria observed between days 10 and 15 could also be due to their natural growth cycle in S2 and C2 cheeses.

Enumerations of TMY and TMAB. The growth of TMY in S2 and C2 cheeses was stimulated by aerobic conditions until day 10 ($P < 0.05$) and then became steady ($P > 0.05$) (Fig. 2). Within the first 10 days, the TMY count increased by approximately 4 to 5 log, thus indicating the increase in pH values of S2 and C2 cheeses. Storage conditions, unlike flavorings, had significant effects on TMAB counts: in anaerobic conditions (associated with low pH), TMAB counts did not change ($P > 0.05$), whereas in aerobic conditions (associated with increased pH), TMAB counts increased by 2 log ($P < 0.05$) (Fig. 3). The flavorings could not be determined as the source of TMAB, since counts of both controls and spice-added samples were not different ($P > 0.05$). Sterilization of most flavorings by gamma irradiation and the addition of the rest of the flavorings in minute amounts accounted for the lack of contribution by the flavorings to TMAB count. The traditional production method renders Sürk cheese prone to contamination. The growth of nonstarter bacteria is usually observed in cheeses *(13, 30)*.

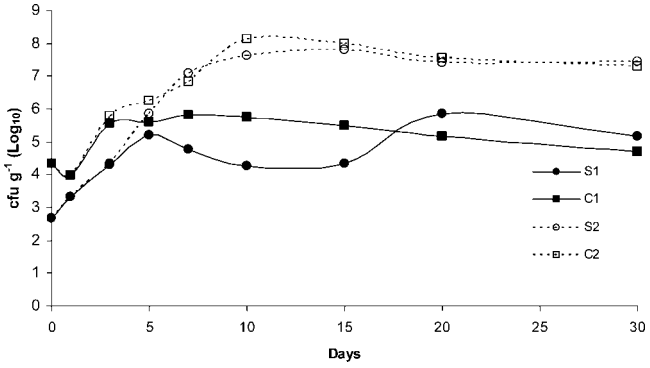

FIGURE 2. *Changes in the number of total molds and yeast in Sürk (S1, S2) and control (C1, C2) cheeses stored aerobically (○, □) or anaerobically (●, ■) for 30 days at room temperature.*

In conclusion, the flavorings used had no immediate inhibitive effect on the survival of *S. aureus.* High acidity of Sürk cheese inhibited the growth of *S. aureus* under anaerobic conditions. However, the growth rate of mold and secondary flora appeared to be a determining factor for the survival of *S. aureus* under aerobic conditions. Since mold growth is desirable for the flavor development of ripened Sürk cheese, elimination of mold during production may be detrimental to the traditional characteristics of the product. Therefore, hygienic precautions are the only criteria of food safety that can be used during the production of Sürk cheese.

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