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EFFECT OF pH ON MICROFLORA OF CIVIL CHEESE DURING REFRIGERATED STORAGE

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Microbial properties, correlation between pH and microbial properties, and the effect of pH adjustment on microflora of Civil cheese during refrigerated storage were examined. The cheeses made from skim milks with a pH of 5.35, 5.30, and 5.25 had higher counts of total aerobic bacteria, yeast and moulds, LAB and psychrotrophic organisms than those made from milks adjusted to higher pH values. A highly negative correlation between pH and total aerobic bacteria, yeast and moulds and LAB was observed in Civil cheese.

Keywords: cheese, storage, microflora, pH adjustment

Civil cheese, an acid curd cheese with rennet added, is a popular cheese variety in Turkey and is generally produced in the eastern Anatolian region of the country. The production method of Civil cheese includes a special plasticizing and kneading treatment of the fresh curd in hot water to have a fibrous structure and melting and stretching properties. Civil cheese is mostly produced in small family businesses for their needs and also by small dairy processing plants for commercial purposes. Civil cheese is consumed fresh or after 2 to 6 months of storage.

In recent years, phenotypic characterization of micro-organisms in many cheeses has been carried out (PAPADEMAS & ROBINSON, 2000; GOBBETTI et al., 2002). These studies helped to design a standardized starter suitable for cheese manufacture from pasteurized milk. The effect of pH or acid development on different properties of cheeses, except microflora, was reported in the literature (LAWRENCE et al., 1993; YAZICI & DERVISOGLU, 2003). The main objective of this research was to study the effects of pH adjustment on microbial flora of Civil cheese. In addition, microflora and interactions between pH and microbial properties of Civil cheese were studied.

1. Materials and methods

Cheeses were manufactured at the Pilot Plant of Food Engineering Department, Ondokuz Mayis University. Whole milk was supplied by the Animal Science dairy farm of the university. Calf rennet was obtained from Pinar Company, Izmir, Turkey.

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1.1. Cheese making

Skim milk from cream separator was heated to 35 °C in a small vat with steam jacket and held at this temperature until the pH reached 5.45 (A1 samples), 5.40 (A2 samples), 5.35 (A3 samples), 5.30 (A4 samples) and 5.25 (A5 samples). Calf rennet (1.67 ml/100 l milk) was then added. After formation of the curd, cooking between 53 and 58 °C was continued until the whey draining from the curd was clear. The curd in the cheese whey was stretched or pulled with a paddle at 70 °C until a proper sheen, smooth, plastic and fibrous body was obtained. It was then removed from the hot water bath and kneaded by hand for 3–5 min. The hot cheese blocks were hung from a clean bar and let stretch down under their own weight. The stretched cheese ends were put up on the bar again. This knitting process was continued until stretching stopped. The long cheese strips were cut into 12-cm pieces, placed in glass jars with 9% brine solution and stored at 4 °C. Fresh cheese samples were analyzed on the first day and the aged cheese samples at the end of the first, second, fourth and sixth months. Three replicates of cheeses were made.

1.2. Microbiological analyses

Total aerobic bacteria (HOUGHTBY et al., 1992) and psychrotrophic micro-organisms (FRANK et al., 1992) were enumerated by using plate count agar (Oxoid) at 32±1 °C for 48±3 h and at 7±1 °C for 10 days, respectively. Yeasts and moulds and proteolytic micro-organisms were enumerated by using potato dextrose agar adjusted to pH 3.5 with 10% lactic acid (Merck) at 25 °C for 5 days, and by using plate count agar (Oxoid) with 100 g l^{-1} skim milk powder (10% wt/wt) at 21±1 °C for 72 h (FRANK et al., 1992), respectively. Acid-tolerant thermophilic lactic acid bacteria were counted by using MRS agar (Oxoid) adjusted to pH 5.5 after incubation for 72 h at 36 °C in an enriched CO_2 atmosphere (KITCHELL & SHAW, 1975; GARCIA et al., 1987). Coliform bacteria (CHRISTEN et al., 1992) were enumerated by using violet red bile agar (Oxoid) at 32±1 °C for 24±2 h. *Escherichia coli* (IDF, 1994) were counted by using lauryl sulfate broth (Merck) at 37 °C for 24–48 h and EC broth (Merck) at 45 °C for 24–48 h.

1.3. Statistical analysis

Statistical analysis of data was performed by ANOVA using Minitab statistical software (MINITAB, 1996). Linear regression analysis was also done to correlate the microbial properties with each other and with pH (KITCHELL & SHAW, 1975). Significant differences between means were determined using Duncan's multiple range test at $P<0.05$ (O'MAHONY, 1986).

2. Results and discussion

Effect of pH adjustment on microflora during refrigerated storage of Civil cheese is presented in Table 1. The total aerobic microbial counts of cheeses significantly decreased throughout the storage period (P<0.05). The cheese samples of A1 and A2 had significantly lower total aerobic counts than A3, A4, and A5 samples throughout the storage period, except the first day (P<0.05). This result indicates that the survival during 4 °C storage of total microbial population present in cheeses derived from skim milks with lower pH was greater than that in cheeses from skim milks with higher pH. The average counts of yeast and moulds significantly increased until the 60th day of storage followed by a significant decrease (P<0.05) and then they did not change significantly for the rest of the storage period (P>0.05). A1 and A2 samples were not significantly different form each other (P>0.05), but were significantly lower in yeasts and moulds than A3, A4 and A5 samples (P<0.05). Because low pH favours the growth of yeasts (BERESFORD et al., 2001), the cheeses made from skim milks with lower pH had higher numbers of yeast and moulds. Lactic acid bacteria (LAB) in Civil cheese were the most dominant organisms throughout the storage time. Counts of LAB significantly increased up to 120 days of storage (P<0.05) and then they did not change. The survival of LAB in cheeses derived from skim milks with the pH of 5.35 to 5.25 was greater than that in cheeses from skim milks with pH of 5.45 to 5.40. Proteolytic micro-organism counts in Civil cheese were found to be between 1.38 and 3.99, with an average of 2.55 $\log_{10}$ CFU g^{-1}. The pH values of cheeses did not significantly affect the counts of proteolytic micro-organisms (P>0.05). The counts of proteolytic micro-organisms significantly increased until the 120th day of storage and then they declined by the end of the storage time (P<0.05). Because casein degradation in Civil cheese is limited compared to other cheeses (YAZICI & DERVISOGLU, 2003), Civil cheese is expected to have low counts of proteolytic micro-organisms that could produce proteolytic enzymes and break down the casein fractions, thereby contributing to the ripening process. The average count of psychrotrophic micro-organisms in Civil cheese was 3.82 $\log_{10}$ CFU g^{-1}, ranging from 2.59 to 4.77. The highest microbial growth was observed in A3 samples and the lowest in A1 samples. Psychrotrophic micro-organism counts significantly increased until the 30th day, remained stable between days 30 and 60, declined significantly until the 120th day, and finally did not change by the end of the storage time. Coliform micro-organisms were detected only on the first and 30th day. A3, A4 and A5 samples contained significantly lower (P<0.05) levels of coliform micro-organisms than A1 and A2 samples. No *E. coli* was detected throughout storage in any of the cheeses tested.

pH values of Civil cheeses were determined on day 1, and after 1, 2, 4 and 6 months of storage. A highly negative correlation (P<0.05) between pH and total aerobic bacteria, yeast and moulds and LAB was observed in Civil cheese. The pH did not have a significant effect on proteolytic and psychrotrophic micro-organisms in Civil cheeses (P>0.05). Since there was only a slight downward tendency in the counts of total aerobic bacteria, yeast and moulds and LAB of Civil cheese throughout the storage time (Table 1), and the pH values of the samples remained almost constant after 30 days of storage, a negative but relatively small correlation coefficient (<0.5) was observed between these variables.

Table 1. Effect of pH adjustment on microbial counts ($\log_{10}$ CFU g^{-1}) during refrigerated storage of Civil cheese[a]

Cheese[b]	Microflora[c]	Storage time, day				
		1	30	60	120	180
A1		7.55±0.11 a	5.05±0.13 ij	5.02±0.07 ijk	4.43±0.22 no	4.29±0.16 o
A2		7.22±0.23 b	5.13±0.14 hij	5.00±0.05 ijkl	4.55±0.04 mno	4.69±0.15 lmn
A3	1	7.25±0.13 b	6.30±0.32 d	5.72±0.30 fg	5.20±0.12 hi	4.83±0.09 jklm
A4		6.73±0.11 c	5.62±0.17 g	5.93±0.09 ef	5.44±0.14 gh	5.42±0.27 gh
A5		6.66±0.41 c	6.25±0.09 d	6.04±0.06 de	5.17±0.13 hi	4.75±0.10 klm
A1		3.08±0.08 defg	2.63±0.47 gh	3.48±0.43 abcde	2.01±0.28 i	1.91±0.18 i
A2		3.02±0.07 efg	3.52±0.30 abcde	3.52±0.39 abcde	2.24±0.16 hi	2.26±023 hi
A3	2	3.32±0.23 bcdef	3.90±0.15 a	4.00±0.22 a	2.71±0.65 gh	3.12±0.22 cdefg
A4		3.02±0.15 efg	3.15±0.27 cdefg	3.82±0.48 ab	3.20±0.46 cdefg	3.33±028 bcdef
A5		2.81±0.22 fg	3.15±0.34 cdefg	3.67±0.26 abc	3.64±0.11 abcd	3.48±0.38 abcde
A1		6.92±0.23 a	4.22±0.24 j	5.20±0.22 fgh	4.15±0.03 j	3.99±0.03 j
A2		6.41±0.24 bc	5.07±0.21 fghi	4.87±0.11 hi	4.18±0.23 j	4.79±0.02 i
A3	3	6.31±0.35 c	5.63±0.44 de	6.17±0.12 c	5.17±0.04 fghi	5.24±0.18 fgh
A4		6.72±0.23 ab	5.79±0.29 d	6.25±0.24 c	5.46±0.14 def	5.03±0.35 ghi
A5		6.33±0.08 c	5.18±0.03 fgh	5.77±0.08 d	5.43±0.20 defg	5.29±0.04 efg

Table 1. (Cont.)

Cheese[b]	Microflora[c]	Storage time, day				
		1	30	60	120	180
A1	4	1.38±0.49 l	2.45±0.22 efgh	3.19±0.07 bcd	3.25±0.10 bc	2.72±0.06 defg
A2		1.49±0.52 kl	2.37±0.22 fghi	2.86±0.15 cde	3.48±0.21 b	2.75±0.04 b
A3		1.95±0.20 ij	2.04±0.21 hij	2.28±0.39 ghij	3.49±0.05 b	2.21±0.12 hij
A4		1.87±0.10 jk	2.20±0.16 hij	2.51±0.20 efgh	3.34±0.27 b	2.83±0.17 cdef
A5		1.47±0.03 kl	1.84±0.46 jkl	2.22±0.39 hij	3.99±0.11 a	3.57±0.48 ab
A1	5	4.14±0.25 abcde	4.15±0.60 abcde	4.46±0.23 abcd	2.59±0.70 i	2.82±0.76 hi
A2		3.84±0.24 cdef	4.52±0.43 abc	4.29±0.24 abcd	2.99±0.05 ghi	2.70±0.17 hi
A3		3.83±0.02 cdef	4.56±0.33 ab	4.37±0.30 abcd	3.50±0.24 efg	3.75±0.17 def
A4		3.84±0.22 cdef	4.15±0.59 abcde	4.77±0.39 a	3.77±0.10 def	3.34±0.06 fgh
A5		4.04±0.25 bcdef	4.41±0.29 abcd	3.81±0.64 cdef	3.84±0.27 cdef	3.06±0.08 ghi

[a] Mean values of three cheeses. Means with same letters within the same category are not significant at P>0.05.
[b] A1, A2, A3, A4 and A5 represent cheeses made from skim milks with a pH of 5.45, 5.40, 5.35, 5.30 and 5.25, respectively.
[c] 1: Total aerobic counts; 2: Yeast and mould counts; 3: lactic acid bacteria counts; 4: proteolytic micro-organism counts; 5: psychrotrophic micro-organism counts.

The highest positive correlation was found between total aerobic bacteria and LAB. The counts of LAB throughout the storage time largely contributed to the total aerobic bacteria of Civil cheeses.

3. Conclusions

The cheeses made from skim milks with a pH of 5.35, 5.30, and 5.25 had increased survival rates for total aerobic bacteria, yeast and moulds, LAB and psychrotrophic organisms. However, pH did not have a significant influence on proteolytic organisms. A negative correlation between pH and total aerobic bacteria, yeast and moulds and LAB was observed.

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