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Microbial Changes on Full and Reduced Fat Edam Cheese during Maturation

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The objective of this research was to determine the microflora of full fat and reduced fat Edam cheese during maturation. Reduced fat (16% fat) and full fat (28% fat) Edam cheese was stored up to 20 weeks at 6°C after manufacture. The cheeses were analyzed for pH, moisture, total solids, fat and protein content. Reduced fat cheese had higher protein and water content than full fat. We determined total bacteria, lactococci, and lactobacilli counts of reduced and full fat cheese during 5 months of ripening. Results revealed that total bacteria counts were about 1.9×10^5/g at one day for reduced fat cheese and 8×10^6/g for full fat cheese. Total counts decreased during maturation in both cheeses. Initial lactococcal counts were about the same in both products.

Keywords: Edam cheese, microflora, reduced cheese

Consumers are much more concerned today about total fat, saturated fat and cholesterol in foods which they eat. Cheese manufacturers are producing low fat cheddar cheese to satisfy this demand. Consumer demand for low fat food has impacted many food processing industries. A significant portion of newly introduced reduced or low fat foods are dairy products and demand for reduced fat cheeses is growing.

Composition, structure, and flavor characteristics of fermented products are determined by the type of starter culture used, type of enzyme addition, incubation and ripening temperature, and ripening microflora (Vanderzant & Splittstoesser, 1992). The microbial flora of natural or processed ripened or unripened cheese is complex (Richardson, 1985). In the microflora of maturing Cheddar cheese there is a sequence of changes in which other species of bacteria, the lactobacilli in particular, replace the initial flora of streptococci (Dawson & Feagan, 1957).

Clark and Reinbold (1967) said that enterococci may constitute about one-half of the low temperature flora of young Cheddar cheese; 56% of the enterococci they found were *Streptococcus durans*. Only 13.0% of the low-temperature flora were lactic group streptococci.

Dovat *et al.* (1971) found that *S. durans* strains produced as much as ten times more acetic acid than the others. Five strains of lactic streptococci produced the lowest quantities of acetic acid.

Poullet *et al.* (1991) studied Casar cheese during ripening. They found that microbial counts in the interior of the cheese were 1.5 log units higher in winter cheeses for total viable counts, lactic streptococci and leuconostocs, and >2 log units higher for total enterobacteria in the 60 days samples.

Clark and Reinbold (1967) isolated 967 microorganisms from commercial cheese samples. This number included 475 enterococci, 148 micrococci, 126 lactic streptococci, and 108 miscellaneous organisms. Foster *et al.* (1957) stated that the organisms found in very young Cheddar cheese were usually lactic streptococci, either *Streptococcus lactis* or *S. cremoris*, often employed in a mixed culture with a species of leuconostoc. They maintained that after the supply of lactose was depleted, the number of streptococci started to decline and lactobacilli began to predominate. The species frequently found in cheese were *Lactobacillus casei* and *Lactobacillus plantarum*.

Kucukoner and Martin (1995) analyzed low-fat and full fat Cheddar cheese to determine total bacteria, streptococci, and lactobacilli counts during maturation. They concluded that there was an increase in lactobacilli counts during ripening, while, there was a decrease in streptococci and in the total count. The fresh cheese curd contained between 10^8 and 10^9 viable starter bacteria per gram (Kucukoner & Martin, 1995). These were inactivated during maturation of the cheese at a rate dependent on the species. Strains of *S. cremoris* tended to die out more rapidly than strains of *S. lactis* (Robinson, 1981).

Haque *et al.* (1997) found that as the cheese aged, full fat Cheddar cheese had higher numbers of lactococci, however, there was a general trend for these counts to decrease over time as indicated by an average of 3 observations. They also stated that the bacterial count on day 1 was similar in low fat and full fat samples, but as the cheese matured the counts changed.

The impact of reduction in fat content of microbial flora is not known. The objective of this study was to determine differences in the microflora of full fat Edam cheese versus reduced the fat variety.

Materials and Methods

Reduced fat cheese (16% fat) and full fat Edam cheese (28% fat) were made by a modified method as follows (Kosikowski, 1982; Kucukoner & Haque, 1997).

On the day of cheese manufacturing, the milk was immediately heated to 32°C and maintained at there. A mixed strain concentrated culture (Mashall's Superstart, Miles-Marschall's

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Dairy Products, Elkhart, IN) was added at the rate of 18 ml/100 kg of milk. Milk was ripened for one h after the starter had been well mixed with cheese milk.

After 60 min, single strength Anatto cheese coloring (Mills Laboratory, Inc., Elkhart, IN) was added at the rate of 20 ml/100 kg of milk. The coloring was diluted 1:15 with tap water prior to addition. Immediately after adding the coloring, single strength rennet (Chr. Hansen's Lab, Inc., Milwaukee, WI) was added at the rate of 11 ml/100 kg of milk. The rennet was diluted 1:15 with tap water prior to addition.

After 30 min, or until a proper coagulum was formed, the curd was cut by the rotation of the stirring and cutting blade assembly. The cut curd was allowed to settle for 10 min at which time the cooking was begun with stirring.

Cooking was started by increasing the temperature gradually from 32°C to 38°C over 30 min. The temperature was held at 38°C for 75 min, while the curd was stirred continously. After titrable acidity of the whey reached 0.17% was drained. The temperature was reduced to 32°C and the curd was packed and cut into small blocks; the temperature was held at 32°C until the titrable acidity reached 0.25%.

The curd was then hooped in 1.6 kg hoops and pressed for 1 h at 15 psi, followed by dressing and second pressing at the same pressure for another 1 h. The cheese thus produced was brine-salted for 48 h at 5°C, then waxed and vacuum packed in a cryovac plastic bag. Aging followed at 6°C for 20 weeks.

Compositional analyses Moisture content was determined by oven drying (at 100°C for 24 h) of a 2 g sample until a constant weight was obtained. Fat content was measured by the Babcock method as described by Marth (1978). The pH was measured using a Fisher Accument Mini pH meter model 640. Cheeses were analyzed for protein with a Labconco semi micro Kjeldahl digestion and distillation unit (Labconco Co., Kansas City, MO) using a modified AOAC method (955.04 AOAC 1990).

Microbial analyses The cheese was analyzed in curd, after cooking one day each month for five months. Cheese sample weight was 11 g from all treatments. Sterile sodium citrate solution (2%) was used as a dilution fluid and a series of dilutions were prepared. A series of decimal dilutions were made as needed to obtain countable plates in the 10^1 to 10^6 range from the original 1:10 dilution obtained from 11 g of cheese in 99 ml citrate buffer. The medium used was Plate Count Agar (Fisher Scientific). Approximately 10 ml of plate count agar was poured into plates, mixed carefully, left to solidify, inverted and incubated at 32°C for 48 h, then counted and the results reported. The recommended medium for this method is potato dextrose agar (PDA) (Marth, 1978). Sterile 10% tartaric acid was used to acidify the medium prior to pouring it into the plates. The pH was adjusted to 3.5 with 10% tartaric acid. Fifteen milliliters of PDA was poured into plates and incubated at 21°C for 5 days. *Lactococcus* and *Lactobacillus*: Medium M 17 (lactose added) (Difco Laboratories, Detroit, MI) was used for isolation and enumeration of lactic acid bacteria (*Lactococcus*). Different dilutions were made from the original 1 to 10 dilutions. Ten milliliters of M17 was poured into plates, mixed, left to solidify inverted and incubated at 37°C for 48 h. The colonies were counted and results recorded. Colonies not changing to yellow were not counted. Lactobacilli were enumerated similarly by plating on LBS (Becton Dickinson and Co., Cockeysville, MD). The plates were incubated for 48 h at 37°C (Vanderzant & Splittstoesser, 1992; Richardson, 1985).

Statistical analysis Results were analyzed by the Statistical Analysis System (SAS, 1988) with three replications. Means were separated by the predicted difference test. Significant differences were determined as $\alpha=0.05$.

Results and Discussion

Compositional analyses Full fat and reduced fat Edam cheese samples were analyzed at the start of the ripening process. The moisture and fat contents of cheese samples showed marked changes as expected. There were no significant differences in the pH or ash content of the cheese as shown in Table 1. The reduced fat cheese had significantly higher protein content than the full fat. Development of pH during ripening showed a change, and by the time of storage there was an increase in pH of both cheese samples.

Microbiological analyses In this research, samples were taken from cheese in curd, after cooking, at one day and there after at 4, 8, 12, 16, 20 weeks. Total bacterial counts were about 1.71 log/g at one day for the reduced fat cheese and 3.0 log/g for the full fat cheese. The levels of total bacteria in the cheeses did change during storage (Table 2), although there were significant differences between samples at the different stages of ripening. Total bacterial count began to increase after cooking and continued until the cheese was 4 weeks old.

Table 1. Composition of reduced fat (16%) and full fat (28%) Edam cheese.

Cheese	Fat	Moisture	Protein	Ash	pH
Reduced fat	$16^{a)}$	$45^{a)}$	$32.1^{a)}$	$3.4^{a)}$	$5.1^{a)}$
Full fat	$28^{b)}$	$39^{b)}$	$25^{b)}$	$3.2^{a)}$	$5.0^{a)}$

$^{a),b)}$, Rows without common superscript differ $p<0.05$.

Table 2. Average of total bacteria and Lactococcus counts determined during storage and the results of the Duncan test (full and reduced fat Edam cheese).

Storage time	n	Total bacteria (CFU log/g)	Lactococcus (CFU log/g)
A	6	$1.39^{e)}$	$3.58^{c)}$
B	6	$2.39^{b)}$	$3.82^{b)}$
C	6	$2.41^{b)}$	$3.99^{a)}$
D	6	$2.46^{a)}$	$2.9^{d)}$
E	6	$1.80^{c)}$	$2.52^{e)}$
F	6	$1.68^{d)}$	$2.07^{f)}$
G	6	$1.19^{f)}$	$1.75^{g)}$
H	6	$1.12^{f)}$	$1.18^{h)}$

$^{a)-h)}$Rows without common superscript differ $p<0.05$. A: In curd, B: After cooking, C: 1 day, D: 4 weeks, E: 8 weeks, F: 12 weeks, G: 16 weeks, H: 20 weeks old cheese samples.

Table 3. Average of total bacteria and Lactococcus counts determined during storage of 20 weeks.

Treatment	n	Total bacteria (CFU log/g)	Lactococcus (CFU log/g)
RF	24	$1.55^{b)}$	$2.43^{b)}$
FF	24	$2.06^{a)}$	$3.01^{a)}$

$^{a),b)}$Rows without common superscript differ $p<0.05$.

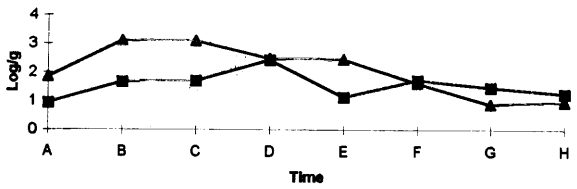

Fig. 1. Total bacterial count from reduced fat and full fat Edam cheese during maturation. A: In curd, B: After cooking, C: 1 day, D: 4 weeks, E: 8 weeks, F: 12 weeks, G: 16 weeks, H: 20 week old cheese samples. ■ reduced fat cheese samples, ▲ full fat cheese samples.

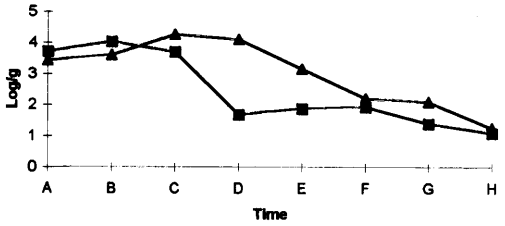

Fig. 2. Lactococci counts from reduced fat and full fat Edam cheese during maturation. A: In curd, B: After cooking, C: 1 day, D: 4 weeks, E: 8 weeks, F: 12 weeks, G: 16 weeks, H: 20 week old cheese samples. ■ reduced fat cheese samples, ▲ full fat cheese samples.

There after that the number of bacteria rose slowly. The total bacteria counts were different between samples and there was also a decrease in bacteria number with storage. The overall total bacteria count was higher in full fat Edam cheese (Table 3) and the results are shown in Fig. 1. Haque *et al.* (1997) found similar results in low fat and full fat Cheddar cheese.

In this study of maturing Edam cheese, the maximum population of starter bacteria occurred immediately after pressing (before salting). The bacterial population at this stage was about 3.71 log/g for reduced fat cheese and 4.26 log/g for full fat Edam cheese. Counts of starter lactococci were high in freshly made Edam cheese, and the number declined during maturation (Fig. 2). There were significant differences between the samples at different stages of ripening. The bacterial counts were 1.1 log/g for reduced fat cheese and 1.25 log/g for full fat cheese at the end of the 5 months of ripening (Table 2). Litopoulou *et al.* (1993) reported that in early ripening, high acid production retarted cheese proteolysis by latococci. Cromie *et al.* (1987) found that streptococcal count rose slightly during ripening at elevated temperatures and fell slightly during ripening at the control temperatures. They also stated that there was no apparent relationship between any bacterial group and an "off" flavor.

Counts of lactobacilli, although none were knowingly included in the cheese culture, started to multiply around 4 weeks and increased slowly during ripening. Law *et al.* (1979) reported that non-starter lactic acid bacteria increased more rapidly at higher temperature. Kucukoner and Martin (1995) found that total counts declined steadily during ripening in low-fat and full fat Cheddar cheese. They also stated that initial streptococci counts in low-fat cheese dropped significantly ($p<0.05$) after 8 weeks, remained about the same through 6 months, and declined again after 9 months. Our findings are in agreement with those reported earlier (Law *et al.*, 1979; Kucukoner & Martin, 1995).

Mold and yeast count was not greatly different in the cheese samples. At the beginning of ripening, mold and yeast numbers were very low, but during maturation increased slightly; these ranging from 1.4-4.6 log/g.

Conclusion

These data indicated few differences in the microflora of full fat Edam cheese versus the reduced variety. It thus follows that the type and amount of enzymes available for hydrolysis and proteolysis in the cheese were about the same. Finally, the addition of certain lactobacilli in starter cultures might improve the flavor development in reduced fat Edam cheese. To produce a reduced fat cheese of acceptable quality, the important structural and flavor role of starter needs to be regulate.

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