Milk Catalase Activity as an Indicator of Thermization Treatments Used in the Manufacture of Cheddar Cheese

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ABSTRACT

Pilot-scale studies were carried out to determine the effect of different heat treatments on catalase activity during the manufacture and maturation of Cheddar cheese. Three trials were conducted to monitor catalase activity using disk flotation and polarographic methods. Cheese was manufactured from raw milk and from milk that had been treated at 60, 65 and 72°C for 16 s using a high temperature, short time heat exchanger. Catalase activity was also determined in samples of commercial milk and in samples of mild, medium, sharp, and extra sharp Cheddar cheeses obtained from different manufacturers in order to verify that the enzyme could be used as an indicator of the type of heat treatment applied to cheese milk.

Catalase activity was present in cheese made from raw milk but was only present at low concentrations in cheese manufactured from thermized milk. However, high catalase activity was observed in commercial samples of sharp and extra sharp Cheddar cheese that was apparently due to the growth of catalase-producing yeasts in the cheese during maturation.

(**Key words**: milk catalase, thermization, Cheddar cheese)

INTRODUCTION

Present regulations governing the manufacture of dairy products state that cheese, including cheese curd, that is not made from pasteurized milk cannot be sold unless it has been stored (at 2°C or above for 60 d or more). At one time, storage under these conditions of cheese made from an unpasteurized source was thought to be equivalent to pasteurization for the reduction of foodborne pathogens. It is now recognized that such a storage treatment does not eliminate foodborne pathogens, such as *Salmonella* spp. and *Listeria monocytogenes*, from certain cheeses (16). Recent work (23) has shown that *Escherichia coli* O157:H7 survives in Cheddar cheese for at least 150 d if present in the cheese milk at numbers of 10^3 cfu/ml (23). Outbreaks of foodborne illness linked to the consumption of raw milk cheese (1, 2, 5, 10, 18, 19, 22) have prompted Health Canada to suggest amendments to the present regulations governing the sale of these products.

Treatments at temperatures from 60 to 63°C give at least a 4-log reduction of *Salmonella* spp. and a 2-log reduction of *L. monocytogenes* in milk (6, 7). In addition, pathogens such as *Salmonella* spp. and *L. monocytogenes* do not grow in hard cheeses (16). Therefore, for certain cheeses, a combination of both thermization treatment and storage is considered to be equivalent to pasteurization. However, no method currently exists to monitor heat treatments applied to milk in the thermization range (63 to 65°C for 16 s). Earlier works (11, 21) have suggested that catalase was inactivated at thermization temperatures, and this suggestion was supported by results obtained from differential scanning calorimetry studies on the enzyme (13). Thus, a study was instigated to determine whether catalase activity could be used as an index of the thermization treatments of milk during cheese manufacture. Two methods of assaying for catalase activity were investigated; the disk flotation assay (4) and the polarographic method (17). The former is a rapid and convenient method that could be easily used in a cheese manufacturing plant, and the latter is a more sensitive technique that requires specialized equipment.

MATERIALS AND METHODS

Milk Samples

Raw milk from the farm bulk tank was collected from the Holstein herd at the Elora Research Station, University of Guelph. Commercial milk samples (500 ml) were collected in sterile bottles from the manufacturing plants and stored at 7°C in ice boxes during transportation to the laboratory.

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Cheddar Cheese Manufacture

Milk (50 kg) was collected from the Elora Research Station and stored overnight at 5°C. Milk was standardized with skim milk powder to a ratio of protein to fat of 0.96. After standardization, between 10 and 15 kg of milk were heated at 60, 65, and 72°C for 16 s using an HTST unit (type HX; APV, Manor Royal, Crawley, United Kingdom). After HTST treatment, the milk was cooled to 31°C before the addition of starter culture.

Lactococcus lactis, *Lactococcus cremoris* (1%, wt/vol; Chr. Hansen's Laboratory Co., Mississauga, ON, Canada), or both, were added as a starter to each of four vats containing raw milk or milk heated at 60, 65, or 72°C for 16 s, respectively, and left to ripen until titratable acidity increased by 0.01%. Calf rennet (diluted 1:30 in distilled water; Chr. Hansen's Laboratory Co.) was added to each vat at a ratio of 2 ml of rennet to 10 kg of milk. Coagulation took place in 0.5 h at 32°C. The temperature was increased at a rate of 1.5°C/min until it reached 39°C. The curd was gently agitated during this cooking stage. The temperature was held at 39°C until the pH reached about 6.1. When the curd pH was between 6.0 to 6.1 and the whey pH was between 6.2 to 6.3, the whey was removed.

The curd was piled along the side of the vats and turned every 15 min until the pH reached 5.4; then the curd was cut and drained further before the addition of salt (1 kg/500 kg of milk). After the salt was well absorbed and the flow of whey had stopped, the curd was ready for hooping. The hoops were lined with a single layer of cheese cloth. Pressing of the curd took place overnight. The cheese blocks were removed from the hoops and vacuum-sealed in clean polyethylene bags. Approximately 700 to 900 g of cheese were obtained from each of the milks. The cheese blocks obtained from each of the different milks were stored at 5°C. This manufacturing process was carried out on three different occasions.

Enzymatic Analysis

Catalase assay using the disk flotation method. Catalase activity was determined in samples that had been collected throughout the cheese manufacturing process (milk, whey, curd, and cheese). Paper disks (8 mm diameter, Whatman 3MM chromatography paper; Whatman, Clifton, NJ) were saturated with 10 μl of the milk or 10 μl of cheese slurry. The slurry was prepared by stomaching (model 400 Stomacher Lab Blender; Seward Medical, London, United Kingdom) 10 g of cheese with 90 ml of phosphate buffer (0.2 *M*; pH 7.0) for 5 min. The disks were immediately dropped into test tubes containing 5 ml of a 3% (vol/vol) solution of H_2O_2 prepared by dilution in a 1×10^{-6} *M* EDTA solution. The elapsed time was recorded between the moment the disk touched the surface of the H_2O_2 solution as it fell toward the bottom of the tube and the time for it to resurface. This interval was found to be inversely proportional to the enzyme concentration in the sample (4, 9).

Catalase assay using the polarimetric method. Catalase activity was also measured polarimetrically (14, 17) using a Clark oxygen electrode in a Gilson 516 oxygraph (Mandel Scientific, Guelph, ON, Canada) equipped with a chart recorder. The reaction was carried out at room temperature (23°C ± 1°C). Calibration of the electrode, before use and several times during the period of use, was done by adding air-saturated water to the reaction chamber with the magnetic stirrer on and by adjusting the sensitivity control of the polarizing circuit to give a full-scale deflection on the chart recorder. The zero oxygen value was set using a sodium sulfite solution. After calibration, 2 ml of either milk or cheese slurry were added directly to the chamber, followed by 100 μl of 3% (vol/vol) H_2O_2. The oxygen evolution was monitored for about 2 min using the chart recorder set at a chart speed of 0.5 mm/s. The initial rate of increase in the concentration of oxygen was calculated from the resulting slope (14, 17).

One unit of catalase is defined as the amount of enzyme required to decompose 1 μmol of H_2O_2/min at pH 7 and 25°C.

Effect of pH on catalase activity in milk. A 5% (vol/vol) solution of lactic acid was prepared and used to alter the pH of 20 ml of raw and heat-treated milk samples to 6.2, 6.0, 5.7, 5.5, and 5.0. The pH was monitored using an Accumet pH meter (model 915; Fisher Scientific, Mississauga, ON). After 10 min of stirring at each pH, the pH was readjusted to 7.0 with 1 *M* NaOH, and the catalase activity was determined using the polarographic method (14).

Microbiological Analysis

Total aerobic plate count. When necessary, 10-fold serial dilutions of milk were prepared using sterile saline (0.85%, wt/vol), and dilutions of the cheese slurry were made with a sterile solution of 2% (wt/vol) sodium citrate. Samples were plated onto milk agar (Oxoid Unipath Inc., Nepean, ON) using a spiral plater (model D; Spiral System Instruments, Bethesda, MD). Plate counts were recorded after incubation for 24 h at 21°C.

Detection and identification of catalase-producing organisms. After the total bacterial count of the samples was recorded, replica plating onto fresh milk agar plates was carried out by making an imprint of the plate on sterile filter paper. The original plates were flooded with a 3% (vol/vol) solution of H_2O_2. Colonies producing catalase could be identified by the associated evolution of oxygen bubbles.

The corresponding colonies were identified on the replica plate, transferred to tryptone soy broth (Difco Laboratories, Inc., Detroit, MI), and incubated at 21°C for 24 h. After purification by repeated subculture, the organisms were identified by Gram stain and an automated microbial identification system (Vitek Junior; bioMérieux Vitek, St. Louis, MO).

Depending on the morphological characteristics of the organism determined by microscopic examination, the appropriate Vitek card (GPI, Gram-positive identification; GNI, Gram-negative identification; or YEAST) was inoculated according to the instructions of the manufacturer. The Vitek Junior selected from its computer database the best match to the biochemical profile of the organism.

RESULTS AND DISCUSSION

Catalase Activity in Milk Samples

Using the disk flotation assay, catalase concentrations in the raw milk samples in April and November were 1.02 and 0.42 U/ml, respectively. No detectable activity was found in milk that had undergone heat treatments of 60, 65, or 72°C for 16 s. The limit of detection for the disk flotation assay is approximately 0.3 U/ml (13).

The average catalase concentration in raw milk that was used for the manufacture of Cheddar cheese, as determined by the polarographic method, was 1.95 U/ml. Approximately 26% of the catalase activity in milk was destroyed by HTST heat treatments at 60°C for 16 s, and 84% of the activity was destroyed when heat treatments increased to 65°C for 16 s. After a heat treatment of 72°C for 16 s, 92% of the enzyme activity was lost in milk. The remaining catalase activity was 1.44, 0.33, and 0.15 U/ml in milks heated for 16 s at 60, 65, and 72°C, respectively.

Analysis of the raw cream and skim milk from one trial indicated that the catalase activity was partitioned with the milk fat. Values for catalase activity of 0.87 and 0.084 U/ml were obtained by the disk flotation assay for the cream and skim milk, respectively.

The disk flotation assay is a convenient method for determining catalase activity, but, because of its high detection limit, the assay has limited application for monitoring enzyme levels in heat-treated milk and milk products. The polarographic method is more sensitive and can reliably detect the low enzyme concentrations that were present in the milk subjected to the heat treatments used in this study.

Catalase Activity in Cheese Samples

The catalase activity in the cheese that was manufactured from raw milk averaged 3.45 U/g when the analysis was performed by the disk flotation method a day after manufacture. When the cheese was examined following 1, 3, 6, and 10 wk of maturation, the catalase activity was below detectable levels (i.e., <3 U/g). Some residual activity remained throughout this storage period, however, as the paper disk floated at least part of the way up the tube in 5 of the 10 tubes used for the assay. No detectable catalase was found in the cheese samples manufactured from any of the heat-treated milks, and no evidence of residual catalase activity was found.

Catalase concentrations in Cheddar cheese during maturation, as determined by the polarographic method, are shown in Table 1. The catalase activity in the raw milk cheese varied between 11.4 to 21.6 U/g, but the mean value (17.01 ± 3.0 U/g) changed little during the 14-wk maturation period. The mean catalase activity throughout storage was lower in the cheeses made from heat-treated milk: 0.032 ± 0.0056 for cheese made from milk treated at 60°C/16 s, 0.007 ± 0.0026 for cheese made from milk heated at 65°C/16 s, and 0.004 ± 0.0022 for cheese made from pasteurized (72°C/16 s) milk. Thus, approximately 55.6, 90.3, and 94.4% of the catalase activity was lost in the cheeses produced from milk that was heated at 60, 65, and 72°C, respectively. As with the raw milk cheeses, the catalase activity in the cheeses made from heat-treated milks showed little variation during the 14-wk storage period (Table 1).

The decrease in enzymatic activity in the cheese may also have been a result of the low pH. To evaluate the relative contributions of pH and heat inactivation on catalase activity, the heat treatments were applied after pH adjustment of the milk. At the natural pH of the milk, a heat treatment of 65°C for 16 s reduced the activity from 2.4 to 0.75 U/ml (Table 2). The catalase activity was further reduced to 0.3 U/ml when the heat treatment was performed at pH 5.5, and this decrease was similar in unheated and heat-treated milks: 56.3% for the unheated milk, 42.2% for the milk heated at 60°C for 16 s, and 60%

TABLE 1. Catalase activity in Cheddar cheese manufactured from raw milk and from milk treated at 60, 65, and 72°C for 16 s.

Storage period	Catalase activity[1]							
	Raw milk		60°C for 16 s		65°C for 16 s		72°C for 16 s	
	(U/g)							
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
1 d	18.45	1.5	10.50	2.1	1.80	0.0	1.05	0.9
2 wk	16.05	4.5	10.35	0.9	2.70	0.6	1.95	1.2
4 wk	18.00	3.3	10.05	0.9	2.55	0.6	1.20	0.9
6 wk	15.75	2.1	9.60	1.2	2.25	0.6	1.35	0.3
8 wk	15.00	3.3	8.70	1.5	2.25	0.6	1.20	0.0
10 wk	19.05	2.1	10.20	4.2	2.25	0.9	1.65	0.6
12 wk	15.00	4.8	8.85	0.9	2.10	1.2	1.05	1.2
14 wk	18.75	3.9	8.40	1.5	1.35	1.5	0.90	0.9

[1]Values represent means of triplicate cheese samples from each of two experiments. Enzyme activity was determined by the polarographic method.

for the milk heated at 65°C for 16 s. Also, the reduction in activity that was produced by heating milk of natural pH at 65°C for 16 s (68.8%) was similar to the reduction in activity that occurred when the heat treatment was applied to milk at pH 5.5 (71.4%). Therefore, the reduction in catalase activity that was observed during the manufacture of Cheddar cheese from heat-treated milk (65°C for 16 s) was primarily due to the heat treatment.

Catalase Activity in Commercial Cheese Samples

Preliminary questioning of four Cheddar cheese manufacturers in southwestern Ontario determined that the heat treatment applied to milk to be used for Cheddar cheese manufacture was in the range of 63 to 65°C for 16 s. To test whether catalase activity was a useful index of heat treatments in this range in a commercial setting, a survey involving sample collection and microbiological and enzymatic analysis was conducted at eight Cheddar cheese manufacturing plants located in northern and southwestern Ontario.

Analyses were carried out during February and March 1993. In the initial study, two plants were selected, and the catalase activity was monitored using the disk flotation assay. The enzyme activity in raw milk from both plants was not significantly different. The activity in the milk from plant A was 1.8 ± 0.06 U/ml, and the corresponding value for plant B was 1.74 ± 0.21 U/ml. Triplicate samples of heat-treated milk (63 to 65°C for 16 s) obtained from the two plants on two separate occasions gave a negative result for catalase, except for one sample from plant A, which had catalase activity of 2.4 U/ml. When the commercial samples of raw milk were heated at 65°C for 16 s in a water bath, no catalase was detected. The catalase activity observed in the heat-treated milk from plant A may have been associated with bacterial contamination after the heat treatment. The bacterial counts in the heat-treated milks from both plants were high (3.2×10^7 cfu/ml for plant A and 4.1×10^4 cfu/ml for plant B), but the value for plant A was so high that it suggests that starter culture may have already been added to this milk. This high bacterial count may explain the detection of catalase by

TABLE 2. The effect of heat treatment on the catalase activity in milks of different pH.

Treatment	Catalase activity[1]									
	pH 5.5		pH 5.7		pH 6.0		pH 6.24		pH 6.65	
	(U/ml)									
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Raw milk	10.5	0.05	13.5	0.14	15.3	0.11	17.7	0.11	24.0	0.09
60°C for 16 s	11.1	0.13	12.6	0.21	14.4	0.16	17.4	0.27	19.2	0.1
65°C for 16 s	3.0	0.02	3.6	0.08	5.4	0.1	5.7	0.07	7.5	0.11

[1]Values are means of three measurements determined by the polarographic method.

TABLE 3. Catalase activity in mild, medium, sharp, and extra sharp Cheddar cheeses manufactured from raw and heat-treated milks and assayed by the disk flotation and polarographic methods.

Cheese sample	Catalase activity[1]		
	Disk flotation	Polarographic method	
		(U/g)	
		$\bar{X}$	SD
From raw milk			
Mild	<6.0[2]	13.8	0.7
Medium	<6.0[2]	14.4	0.1
Sharp	<6.0[2]	12.6	0.21
Extra sharp	<6.0[2]	9.0	0.04
From heat-treated milk			
Mild	ND[3]	1.2	0.04
Medium	ND	2.4	0.05
Sharp	<6.0[2]	12.6	0.21
Extra sharp	<6.0[2]	6.6	0.17

[1]Values are means of three analyses.

[2]A trace amount of catalase activity was present; the disk floated only partly up the tube but did not reach the top.

[3]Activity not detectable.

the disk flotation method. Charbonneau et al. (4) determined the production of catalase by 13 different species of bacteria, and they found that the enzyme was only detectable when total bacterial counts had reached 1×10^6 cfu/ml of culture. When the incubated plates from plant A were flushed with H_2O_2, some colonies produced gas, thus confirming the presence of catalase-producing bacteria.

Analyses were also performed on 450-g samples each of mild, medium, sharp, and extra sharp Cheddar cheese obtained from a local supermarket and manufactured from heat-treated milk. Samples of raw milk Cheddar cheese (450 g) were obtained from a local health store. The results of triplicate analysis that were obtained with the disk flotation and polarographic methods are presented in Table 3. Catalase concentrations for the Cheddar cheese that was manufactured from raw milk were generally lower than those for the raw milk cheese in the pilot-scale experiments. Low concentrations of catalase might have been present in the milk used for the manufacture of the commercial cheeses. The differences may also have arisen because of changes in catalase activity during the maturation process, as the manufacturing date of the commercial raw cheese samples was not known.

When the polarographic assay was used, the catalase activity increased in sharp and extra sharp Cheddar cheeses that were made from the heat-treated milk (Table 3). To confirm that this increase was real, additional samples of cheese from other manufacturers were examined. The results show that the catalase activity did increase ($P < 0.01$) in sharp and extra sharp Cheddar cheeses compared with the activity in mild cheeses (Table 4). Catalase activity was higher ($P < 0.05$) in extra sharp cheeses than in medium Cheddar cheeses. However, in cheeses from plant H, the catalase activity remained low at all stages of maturation. For cheeses from plants E and F, catalase activity in mild cheese was higher than that in cheeses produced at the other plants.

The high catalase activity that was detected in the mature cheeses manufactured from thermized milk was unexpected and may have been the result of lower heat treatments applied to milk used for the

TABLE 4. Catalase activity, measured by the polarographic assay, in mild, medium, sharp, and extra sharp Cheddar cheeses produced in eight plants in Ontario.

Plant	Catalase activity							
	Mild		Medium		Sharp		Extra sharp	
	(U/g)							
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
A[1]	1.2	0.1[3]	11.1	0.15	8.4	0.2	16.5	0.35
B[1]	0.9	0.1	0.9	0.15	14.1	0.21	15.0	0.25
C[2]	2.7	0.04	20.4	0.2	21.6	0.3	18.6	0.2
D[2]	0.9	0.01	1.2	0.01	0.9	0.01	15.0	0.15
E[2]	9.6	0.32	5.4	0.05	20.4	0.28	16.2	0.19
F[2]	9.3	0.5	6.3	0.34	15.6	0.65	7.5	0.03
G[2]	2.4	0.18	4.5	0.15	1.2	0.01	10.8	0.17
H[2]	0.9	0.01	2.7	0.03	2.7	0.05	3.0	0.04
Overall	3.5[c]		6.6[bc]		10.6[ab]		12.8[a]	

[a,b,c]Means with no common superscript letter differ ($P > 0.05$).

[1]Mean of two separate analyses performed on triplicate samples.

[2]Mean of one analysis performed on triplicate samples.

TABLE 5. Mean temperature used to process milk used in the manufacture of Cheddar cheese.

Cheese type	Processing temperature[1]		Range
	(°C)		
	$\bar{X}$	SD	
Mild	73.0	1.1	72–74.4
Medium	67.1	4.5	62.7–73.3
Sharp	66.2	3.5	62.7–72
Extra sharp	66.2	3.5	62.7–72

[1]Values are the means of data from six processing plants.

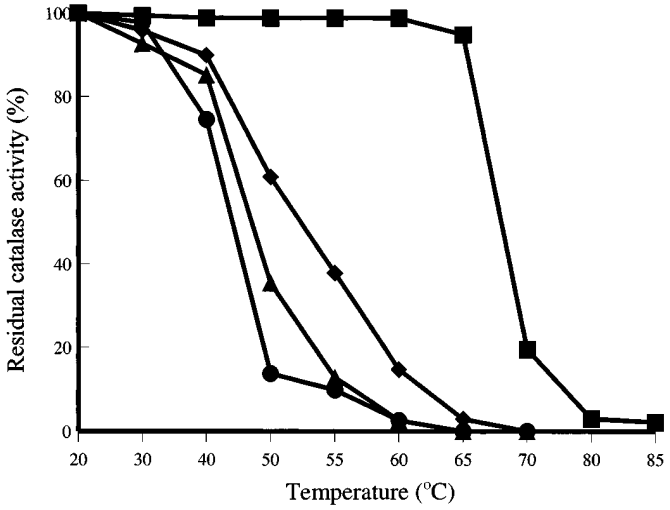

Figure 1. Effect of heat treatment for 16 s at different temperatures on the activity of catalase from *Pseudomonas putida* (●), *Pseudomonas fluorescens* (▲), *Pseudomonas aeruginosa* (■), and bovine milk (♦).

manufacture of sharp and extra sharp Cheddar cheese, reactivation of the inactivated enzyme, heat-resistant catalase arising from bacteria commonly associated with milk, or contamination after heat treatment by catalase-producing microorganisms.

From the responses to the questionnaire sent to participating cheese plants, it was clear that, for five of the six processors that replied, the processing temperatures applied to milk used to make sharp and extra sharp Cheddar were lower than those used in the manufacture of mild and medium cheeses (Table 5). However, the increase in catalase activity that occurred in the sharp and extra sharp cheeses could not be explained solely on this basis. For four of six respondents, the heat treatment used in the manufacture of the aged cheeses was above 65°C, the temperature at which enzyme activity decreased significantly.

There was no evidence that catalase was reactivated following denaturation at 65°C during subsequent storage of the enzyme solution at both 4 and 21°C (13). If the increase in activity during the storage of the cheese was due to reactivation, the response would be expected to be similar in all cheese produced from milk heated at similar temperatures, but such was not the case (Table 4).

Some concern may be raised because many of the common bacterial contaminants of milk are positive for catalase and could interfere with the analysis of the native enzyme in milk and cheese. The production of bacterial catalase varies with the species tested. *Salmonella*, *Shigella*, and *Escherichia* spp. are very similar in their ability to produce catalase, but *Bacillus megaterium* produces higher concentrations of catalase than *Bacillus cereus* (4). Studies have shown that cod muscle catalase was totally inactivated at 50°C for 10 min, but catalase activity from *Micrococcus* spp. and *Pseudomonas* spp. remained unaltered after that particular heat treatment (8).

The thermostability of catalase from three species of *Pseudomonas*, a common contaminant of milk, was determined. The results showed that the temperature that was required for inactivation of bacterial catalase and catalase from bovine milk (15) are similar (Figure 1). Less than 10% residual activity was found at temperatures higher than 60°C for the catalases from bovine milk, *Pseudomonas fluorescens* and *Pseudomonas putida*. However, the catalase from *Pseudomonas aeruginosa* remained active even after heat treatments of 85 to 90°C for 16 s.

A study on lipolytic and proteolytic activity of bacteria isolated from blended raw milk determined that *Pseudomonas* spp. were the predominant bacterial type, constituting about 65 to 78% of the total psychrotroph population in blended raw milk samples (20). An average of 43% of these isolates were of the fluorescent type. The group containing Enterobacteriaceae, *Acinetobacter* spp., and Gram-positive microorganisms accounted for more than 5% of the psychrotrophic bacteria that were isolated. Previous studies have also determined that bacterial numbers must be in excess of 1×10^6 cfu/ml before detectable concentrations of catalase are synthesized (4, 21), and the bacterial levels in raw milk from plants in the present study ranged from 1.8×10^4 to 8.5×10^5 cfu/ml (Table 6). This level, together with the heat resistance data for bacterial catalase from the predominant microorganisms that were present in raw milk, suggests that the contribution to catalase activity in heat-treated milks from bacterial contamination of the milk prior to heat treatment may be minimal.

TABLE 6. Residual catalase activity and total aerobic plate counts for commercial milk samples from four cheese processing plants.

Milk sample	Residual catalase activity[1]	Total aerobic count[2]
	(%)	(cfu/ml)
Plant A		
Raw	100	1.2×10^5
Heated, 63°C for 16 s	42.2	7.6×10^2
Heated, 72°C for 16 s	3.6	$<2.0 \times 10^1$
Plant B		
Raw	100	6.1×10^5
Heated, 65°C for 16 s	84.2	5.6×10^4
Heated, 72°C for 16 s	5.2	1.4×10^2
Plant C		
Raw	100	1.8×10^4
Heated, 62.7°C for 16 s	88.0	7.2×10^3
Heated, 72°C for 16 s	12.6	1.4×10^2
Plant D		
Raw	100	8.4×10^5
Heated, 66°C for 16 s	52.6	1.7×10^3
Heated, 72°C for 16 s	8.7	1.6×10^2

[1]Catalase activity determined by the polarographic method and assays were performed in triplicate.

[2]Plate counts were performed in duplicate.

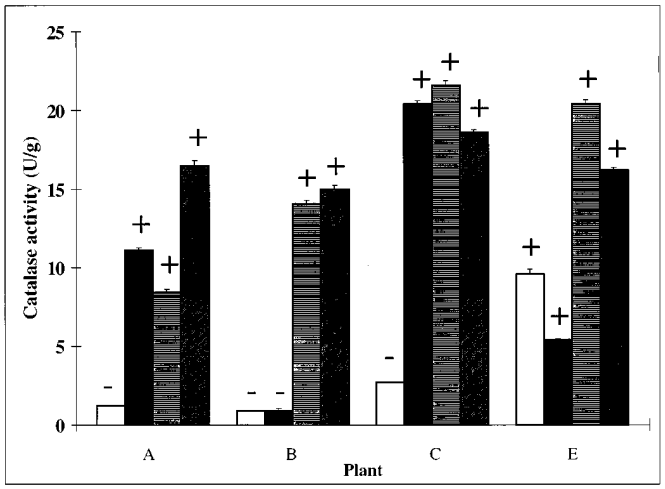

Figure 2. Catalase activity in mild (white bar), medium (black bar), sharp (light gray bar), and extra sharp (dark gray bar) Cheddar cheeses. A minus sign above a bar indicates cheeses from which no catalase-producing organisms were isolated, and a plus sign indicates those cheeses that were contaminated with catalase-producing microorganisms.

To investigate the possibility of contamination after heat treatment, microbiological analysis was carried out on the milk (Table 6) and cheese samples that were obtained from four plants. There was little evidence of postpasteurization contamination of the milk. The low counts observed in the heat-treated milks were due to the presence of microorganisms capable of surviving the heat treatment. When the sharp and extra sharp cheeses were examined, the high concentration of catalase occurring in these cheese samples was due mostly to contamination by catalase-producing yeasts.

Medium, sharp, and extra sharp cheese samples from plants A, B, C, and E were chosen to be plated because of the high concentrations of residual catalase found in these cheeses. Catalase-positive colonies were detected, and their presence correlated well to the high levels of catalase found in these samples (Figure 2). These colonies were isolated and identified by morphological characteristics and by an automated microbial identification system (Vitek Junior; bioMérieux Vitek).

The predominant catalase-producing microorganisms that were present in the sharp and extra sharp cheese samples were yeast, identified as *Candida famata* and *Rhodotorula rubra*. The medium Cheddar cheese samples were contaminated with the same species of yeast but in lower numbers. The Gram-negative bacteria, *Flavobacterium indologenes* and *Pseudomonas vesicularis*, were also prevalent. The Gram-positive bacteria, *Staphylococcus epidermidis* and *Staphylococcus simulans*, were also detected (Table 7).

None of the starter cultures used in the manufacture of Cheddar cheese produced catalase. The

TABLE 7. Catalase-producing organisms isolated from commercial samples of Cheddar cheeses.

Organism	Medium	Sharp	Extra sharp
Gram-positive	*Staphylococcus simulans* *Staphylococcus epidermidis*	None	None
Gram-negative	*Flavobacterium indologenes* *Acinetobacter lwoffi* *P. vesicularis*	*F. indologenes*	*F. indologenes* *Pseudomonas vesicularis*
Yeast	*Rhodotorula rubra*	*Candida famata* *Candida glabrata*	*C. famata* *Candida krusei* *R. rubra*

microflora of Cheddar cheese is composed of the starter bacteria and nonstarter bacteria present in milk that may have survived the heat treatment or gained access as contaminants after heat treatment. Griffiths et al. (12) determined that the numbers of enterobacteriaceae were considerably reduced after thermization, although the numbers of pseudomonads were higher in the heated milk. Chapman and Sharpe (3) described the types of microorganisms that were found in cheese as possibly being contaminants that gained entry before or after heat treatment. Among those listed identified were lactobacilli, pediococci, leuconostoc, staphylococci, micrococci, enterococci, coliforms, pseudomonads, and achromobacteria. The results of the present study are in agreement with the data of Chapman and Sharpe (3), and the microorganisms found in the cheese were those that generally die out during cheese ripening. Thus, evidence points to contamination after heat treatment of these samples. The presence of yeasts, which are not a common contaminant in dairy products because they do not ferment lactose in general and grow comparatively slowly, provides additional evidence of contamination after heat treatment.

CONCLUSIONS

Catalase activity cannot be used as a reliable index of prior milk heat treatment for commercial cheese unless the storage history of the product is known.

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