THE INFLUENCE OF ENVIRONMENT AND PROCESSING ON SPOILAGE ORGANISMS IN COTTAGE CHEESE[1]

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(Received for publication April 20, 1956)

Microbiological analyses were performed on samples taken at various stages of processing of 17 vats of cottage cheese manufactured in 12 commercial plants. In 10 vats of fresh cheese whose shelf-life ranged from 5 to 10 days, the logarithmic averages of the counts of coliform, lipolytic and proteolytic bacteria were 50, 1,298 and 1,967 percent greater than the corresponding averages of 7 vats whose shelf-life ranged from 13 to 16 days. Spoilage organisms were found in water, coagulator, starter, air, contaminated equipment and improperly pasteurized milk and cream. Several water supplies contained high populations of lipolytic and proteolytic organisms, but were free of coliform bacteria which are commonly used as an index of acceptability for water supplies.

In recent years numerous workers have reported on the types of organisms responsible for spoilage in cottage cheese. Deane and Nelson (6) examined 79 samples from 25 commercial plants for yeast, mold, coliform, proteolytic and lipolytic organisms. Poor keeping quality at 21° C. was associated with mold counts in excess of 100 per g. and poor keeping quality at 6° C. was associated with proteolytic and lipolytic bacteria counts in excess of 100 per g. Elliker (7) and Parker *et al.* (12) reported that three species of bacteria, *Pseudomonas fragi, Pseudomonas viscosa* and *Alcaligenes metalcaligenes* are responsible for much of the slimy, gelatinous defect of cottage cheese. These workers isolated the above organisms from equipment, creaming mixtures, plant dust and water supplies. Collins (3) reported that prolonged incubation permitted cultures of *Ps. fragi, Ps. viscosa* and *Alc. metalcaligenes* to cause surface spoilage of cottage cheese at initial pH values as low as 4.6 and at temperatures as low as 3.5° C. Harmon and Smith (8) reported on the relationships between pH and the type of organisms dominating cottage cheese spoilage. Davis and Babel (5) found that cottage cheese curd washed with suspensions containing from 2,000,000 to 120,000,000 bacteria per ml. retained from 0.0093 to 0.40 percent of the organisms. The temperature of incubation had a greater effect on the rate of slime formation than did the number of bacteria in the wash water. Lyons and Mallmann (10) enumerated the coliforms in 150 samples of cottage cheese from 8 plants. Line samples from 6 plants contained coliforms at all sampling points. Sixty percent of the fresh packaged cheese from all plants contained coliforms.

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The data herein reported were secured to determine the source and numerical frequency of each of the various groups of organisms detrimental to the keeping quality of cottage cheese. The relationship was determined between keeping quality and temperatures at critical points in the processing and storage of cottage cheese.

EXPERIMENTAL PROCEDURE

Twelve commercial cottage cheese plants were visited. The complete manufacturing procedure of 17 vats of cheese was observed and recorded. Samples were taken of all ingredients and of the cheese at each of several designated stages of the manufacturing process. Swabs of the vats and handling equipment were made according to Standard Methods (1). The samples were promptly refrigerated and returned to

[1]Mich. Ag. Exp. Sta. Journal Article No. 1904

the laboratory for microbiological and chemical analyses.

The cheese samples were prepared for analyses by mixing 11 g. of cheese and 99 ml. of buffered sterile water for two minutes in a Waring blendor jar. The Waring blendor jars were sterilized by soaking for one hour with a 500 p.p.m. chlorine solution and rinsing with sterile distilled water. Control plates from the rinsings of jars prepared in this manner seldom showed any growth and never more than two colonies. All samples of cheese and raw materials were examined for coliform, lipolytic, proteolytic, yeast and mold organism populations. Total counts were performed on swabs, rinses from containers and on all ingredients except starter. Coliform, yeast, mold and total counts were performed according to Standard Methods (1). Lipolytic organisms were determined by the method of Long and Hammer (9). Proteolytic bacteria were enumerated on a medium containing 0.2 percent peptone, 1.5 percent agar, and 2 percent sterile skim milk added at the time the plates were poured. Air borne contamination was determined in the packaging and manufacturing areas by exposure of poured plates of tryptone glucose yeast agar and acidulated potato dextrose agar, according to Standard Methods.

One ml. or 1 g. portions of all of the samples collected were inoculated into cottage cheese which had been sterilized by autoclaving. The inoculated cheese was held four days at 21° C. to observe the type of deterioration.

Creamed cottage cheese samples were scored organoleptically, stored at 42° and 50° F. and observed for shelf-life. The pH was determined on the dry curd, creaming mixture, and on the fresh and spoiled cheese.

RESULTS

Spoilage organisms in cottage cheese at various stages of manufacturing. All of the vats of cheese observed in this study were made by the "short set" with the incubation times and temperatures varying from 4 to 6 hours and 86° to 90° F., respectively. Since this temperature is favorable for most contaminants, it is not surprising to find significant populations in the cut curd. The range of the various counts (shown in Table 1) is particularly significant and indicates that at this stage of manufacture, contaminants may be completely absent or may have attained sufficient numbers to represent a quality hazard. Most of the vats of cheese were cooked to about 120° F. which resulted in considerable reduction in coliforms, but was not effective in destroying lipolytic, proteolytic, yeast or mold organisms. The washing operation caused a substantial increase in the average counts of lipolytic and proteolytic bacteria, which is attributed to the high populations of these organisms in some of the water supplies. The range of counts of each of the different types of spoilage organisms in the packaged creamed cottage cheese illustrates the wide variation in the microbiological condition of the freshly made product and establishes the fact that it is possible to produce cheese containing very few contaminating organisms. Data in Table 1 also show the range and logarithmic average of the counts of various organisms in the cheese held at 50° F. until spoiled and the counts of duplicate samples held at 42° F. for the same length of time. The advantage of the lower storage temperature in reducing development of organisms is obvious.

Data in Table 1 indicate the results of microbiological analyses of "line samples" from 12 commercial plants. The minimum and maximum counts and the logarithmic average of the counts of each group of organisms determined in the line samples are indicated.

The data show that the pasteurized milk and cream represented a principal source of all types of spoilage organisms determined. Davis and Babel (5) showed that the organisms usually responsible for slime formation in cottage cheese are destroyed by pasteurization. The lack of adequate plant sanitation represented a source of contamination in several plants. Standard Methods (1) recommends that the total swab count of plant equipment should not exceed 12.5 organisms per sq. in. In several of the plants studied swab counts made on sanitized surfaces of vats and equipment revealed less than one organism per sq. in. However, the logarithmic average count on the vats and other equipment were 54 and 38 organisms per sq. in., respectively. No official recommendations exist as to the tolerance of coliform, lipolytic, proteolytic, yeast and mold organisms, but since these types are capable of spoiling cottage cheese, it seems desirable to strive for a degree of plant sanitation which will preclude the presence of these organisms on plant equipment.

In most instances, the coagulator was not a significant source of undesirable organisms, but a few samples possessed high lipolytic, proteolytic and/or yeast and mold populations. A few starter samples and several empty cartons selected at random were found which contained yeast, mold and lipolytic organisms. No coliform bacteria were found in the starters or rinses from the cartons.

Air borne contamination in cottage cheese plants. Data in Table 2 show the yeast, mold and total counts determined on standard agar and potato dextrose agar plates exposed for 15 minutes in the manufacturing

TABLE 1 — MICROBIAL POPULATIONS OF "LINE SAMPLES" FROM 17 VATS OF COTTAGE CHEESE IN 12 COMMERCIAL PLANTS

Type of sample	Micro-organism counts per g. or ml.									
	Total		Coliform		Lipolytic		Proteolytic		Yeast and mold	
	Range	Log. ave.	Range	Log. ave.	Range	Log. ave.	Range	Log. ave.	Range	Log. ave.
Vat swab	<1 to 13,000	54	<1 to 240	1	<1 to 1,900	18	<1 to 1,100	10	<1 to 6,500	6
Equipment swab	<1 to 11,300	38	<1 to 1,400	2	<1 to 3,600	11	<1 to 5,600	24	<1 to 75	3
Coagulator	<1 to 50,000	46	<1	<1	<1 to 3,400	2	<1 to 10,000	9	<1 to 38,000	2
Wash water	<1 to 14,400	43	<1 to 4	<1	<1 to 1,800	5	<1 to 15,000	17	<1 to 25	2
Carton rinse	<1 to 2,400	290	<1	1	<1 to 620	30	<1 to 200	10	<1 to 600	28
Pasteurized milk	500 to 82,000	19,000	<1 to 1,080	4	10 to 5,100	200	2 to 8,000	140	<1 to 100	5
Starter	*	*	<1 to 50	2	<1 to 500	27	<1	<1	<1 to 100	9
Cut curd	*	*	<1 to 9,200	29	<1 to 16,000	140	<1 to 1,500,000	330	<1 to 3,000	11
Curd after draining whey	*	*	<1 to 90	2	10 to 4,000	130	<1 to 50,000	140	<1 to 35,000	20
Washed curd	*	*	<1 to 100	1	40 to 9,000	330	<1 to 300,000	650	<1 to 5,000	21
Cream	100 to 134,000	6,600	<1 to 1,400	14	10 to 30,000	180	<1 to 30,000	320	<1 to 800	14
Packaged cheese	*	*	<1 to 210	7	20 to 36,000	240	to 1,500,000	430	<1 to 2,000	36
Cheese held at 50° F. until spoiled	*	*	<1 to 5,300,000	4,000	1,000 to 2,400,000,000	4,300,000	100 to 1,300,000,000	1,600,000	20,000 to 1,200,000,000	650,000
Cheese held equal time at 42° F.	*	*	<1 to 618,000	200	1,000 to 150,000,000	480,000	100 to 900,000,000	460,000	1,000 to 4,000,000	49,000

*Not determined

TABLE 2 — AIR-BORNE MICRO-ORGANISM POPULATIONS APPEARING ON 15-MINUTE EXPOSURE PLATES IN 12 COMMERCIAL COTTAGE CHEESE PLANTS

	Plates exposed in					
	Cheese manufacturing area			Cheese packaging area		
Plant source	Total	Yeast	Mold	Total	Yeast	Mold
A	13	0	0	64	2	1
C	265	2	11	*	*	*
E	7	2	1	140	2	5
G	17	0	1	58	1	1
R	82	3	4	99	0	4
T	99	2	5	192	5	9
V	290	0	6	153	2	5
Y	181	0	0	84	6	4
AA	44	2	2	*	*	*
DD	99	23	9	9	0	5
EE	46	1	0	85	5	6
FF	83	14	13	64	2	3
Logarithmic average	62.0	2.3	2.5	76	2.2	3.7
Arithmetic average	102.0	4.1	4.3	95	2.5	4.3

*Not determined

and in the packaging areas of commercial cottage cheese plants. The variations in total count are considerably greater than the variations in yeast and molds. The results indicate that the air in all plants represents a potential source of contamination and emphasizes the need to minimize the intervals during which products are exposed.

Contamination from line samples in cottage cheese plants. The "line samples" secured from each of the 12 plants were inoculated into 25 g. portions of cottage cheese which had been sterilized by autoclaving in petri dishes. The inoculated cheese samples were incubated for four days at 21° C. The type of spoilage appearing in the sterile curd is indicated in Table 3. Nine "line samples" were secured from each plant and the number of these "line samples" causing spoilage varied from one to six. Only one sample of pasteurized milk, three samples of cream and four swabs of equipment failed to cause spoilage when 1 ml. portions were inoculated into the sterilized curd. Only one sample of starter produced spoilage in the sterile curd.

Temperatures at critical points in the manufacture of cottage cheese. In each plant the temperatures were determined at various critical stages of manufacturing the cheese and are recorded in Table 4. The plants are divided into two groups, according to the shelf-life of the cheese manufactured therein. Data show that the average temperatures at the critical points in processing and packaging cottage cheese were from

TABLE 3 — TYPE OF SPOILAGE OCCURRING WHEN STERILE COTTAGE CHEESE WAS INOCULATED WITH LINE SAMPLES FROM 12 COMMERCIAL PLANTS AND HELD FOUR DAYS AT 21° C.

Source and nature of inoculating material	Type of spoilage in sterilized cottage cheese inoculated with line samples from plants											
	A	C	E	G	R	T	V	Y	AA	DD	EE	FF
Control	No fermentation											
Equipment												
Carton rinse	N	N	N	P	P	N	M	N	N	N	M	N
Equipment swab	N	N	M, P	N	P	M	M, P	F	N	P	M	N
Vat swab	N	M	S	N	M	N	N	N	N	N	P	N
Products												
Coagulator	N	N	M	P	N	N	N	M	P	M	N	N
Cream	P	N	N	P	P	P	P	N	N	P	P	P
Milk	P, Y	N	P	S, Y	N	F, Y	P	P	F	P	M, P	S
Starter	N	N	N	N	N	N	N	N	F	N	N	N
Whey	N	N	N	M, Y	M	Y	N	N	N	M	N	N
Water	N	N	N	N	P	P	M	N	P	N	N	P

Legend:

F = fruity
M = moldy
N = none or normal
P = putrid
S = sour
Y = yeasty

TABLE 4 — TEMPERATURES AT CRITICAL POINTS IN MANUFACTURING COTTAGE CHEESE IN 12 COMMERCIAL PLANTS

Plant source	Temperature in deg. F.									
	First water	Last water	Washed curd	Packaged cheese	Packaging room	Cheese in storage		Storage vaults		Shelf-life in days
						Bulk	Packaged	Bulk	Packaged	
Cottage cheese with shelf-life ranging from 5 to 10 days at 50° F.										
A	52	52	79	52	70	42	40	38	38	10
C	48	48	52	*	*	43	42	41	41	7-8
E	53	53	60	62	74	46	44	43	43	9
G	55	40	49	48	64	47	45	44	44	5
R	45	35	50	50	72	51	37	50	35	10-10
V**	54	42	51	48	64	46	40	44	37	9
Y	58	58	62	44	*	42	44	42	42	8
EE	53	53	59	66	70	44	43	42	42	8
Average	52	48	58	53	69	45	44	43	40	8.4
Cottage cheese with shelf-life ranging from 13 to 16 days at 50° F.										
T	85	44	51	56	66	39	46	34	34	13
V**	54	42	51	48	64	46	40	44	37	13-13
AA	52	52	50	*	*	40	*	33	33	14
DD	56	42	49	44	*	*	43	42	42	16-16
FF	70	48	51	58	68	43	40	41	41	16
Average	63	46	50	52	66	42	42	39	37	14.4

* not determined

** samples from plant V occurred in both groups.

2° to 8° F. lower in the vats of cheese with the longer shelf-life.

Relationship between organoleptic grade, pH, microbial populations and shelf-life of commercial cottage cheese. Data in Table 5 show the initial counts of coliform, lipolytic, proteolytic, yeast and mold organisms in packaged samples from 17 vats of cheese. At 50° F. ten samples had a shelf-life ranging from 5 to 10 days and seven samples had a shelf-life of 13 to 16 days. In the fresh cheese the logarithmic averages of the counts of coliform, lipolytic and proteolytic organisms were 50, 1,298 and 1,967 percent, respectively, greater in the group of samples with shelf-life between 5 and 10 days than in the samples with shelf-life between 13 and 16 days. The yeast and mold counts were 104 percent greater in the samples with the longest shelf-life. This relationship may be attributed to the fact that the samples with the longer keeping quality possessed a lower average pH and yeasts and molds are more acid tolerant than the other groups of organisms.

When initially analyzed, seven of the 17 samples contained less than one coliform bacterium per g., two contained less than one yeast and mold per g. and seven contained maxima of 100 organisms per g. in the lipolytic and proteolytic groups. The coliform and yeast and mold counts exceeded 100 per g. in one and three samples, respectively. The lipolytic and proteolytic counts exceeded 10,000 per g. in one and seven samples, respectively.

The initial score, initial and terminal pH, shelf-life and type of spoilage are shown by the data in Table 5. The initial score averaged slightly higher in the group of samples with the longer shelf-life. The pH of both the fresh and spoiled samples averaged lower in the group with better keeping quality. The benefit of good refrigeration is indicated by the fact that the average shelf-life of all samples was 51 percent longer at 42° F. than at 50° F. The keeping quality of the group with the shorter shelf-life was 62 percent longer at 42° than at 50° F.; in the group with the longer shelf-life the keeping quality was extended 43 percent at the lower temperature. Slime formation was the more common type of spoilage in the group of samples with the higher pH and short shelf-life; moldiness and high acidity were the common defects in the group with the lower pH and longer shelf-life.

DISCUSSION

Proper pasteurization destroys most of the organisms which were enumerated on the differential media used in this investigation. High counts occurring in milk samples taken at the cheese vat and in cream samples taken at the time the cheese was being

TABLE 5 — RELATIONSHIP BETWEEN ORGANOLEPTIC GRADE, pH, MICROBIAL POPULATIONS AND SHELF-LIFE OF PACKAGED SAMPLES OF 17 VATS OF COTTAGE CHEESE FROM 12 COMMERCIAL PLANTS

Plant source	Initial score	pH		Organism counts				Shelf-life in days	
		Fresh	Spoiled	Coliform	Lipolytic	Proteolytic	Yeast & mold	50° F.	42° F.
10 vats with shelf-life ranging from 5 to 10 days at 50° F.									
A	39	4.62	4.88	0	100	10	0	10	20
C1	39	4.81	5.12	33	1,800	15,000	*	7	12
C2	39	5.15	5.12	39	1,200	2,300	*	8	9
E	41	5.32	5.15	10	110	100	40	9	13
G	39	4.72	4.70	210	36,000	28,000	50	5	6
R1	39	5.10	5.20	10	400	300,000	230	10	13
R2	40	5.10	4.69	0	2,000	1,500,000	30	10	20
V3	39	5.21	5.25	0	1,000	300	10	9	13
Y	40	4.92	4.80	4	60	50	100	8	15
EE	39	5.30	5.01	20	6,000	11,000	20	8	15
Average	39.4	5.03	4.99	9	1,300	3,100	27	8.4	13.6
7 vats with shelf-life ranging from 13 to 16 days at 50° F.									
T	40	4.90	4.67	0	100	100	190	13	20
V1	40	4.81	4.86	0	50	10	20	13	17
V2	39	4.99	4.85	0	20	62,000	100	13	20
AA	40	4.62	4.42	0	*	0	2,000	14	18
DD1	41	5.37	5.15	40	100	1,000	20	16	23
DD2	40	5.21	4.92	50	1,000	34,000	0	16	23
FF	41	4.96	4.82	100	70	0	100	16	23
Average	40.1	4.98	4.81	6	93	150	55	14.4	20.6
Average of all samples	39.7	5.01	4.92	7	240	430	36	10.9	16.5

* Lost

creamed indicated either unsatisfactory pasteurization or post-pasteurization contamination.

The coliform bacteria and yeasts and molds are retarded at the temperatures at which cottage cheese is normally held. However, these temperatures are less adverse to members of the genus *Pseudomonas* which includes many of the lipolytic and proteolytic organisms commonly encountered in dairy products. According to Bergey's Manual (2), the optimum temperatures for growth of most of these organisms vary from 10° to 25° C., and growth is retarded at 5° C. (40° F.). The predominant defects which developed in the cheese samples held at 42° F. were mold, high acid and also stale or bitter flavors suggestive of enzyme activity. At 50° F. spoilage usually was caused by the formation of pigment and surface slime or gelatinous curd.

The amount of air-borne contamination varied considerably among plants, but there was little correlation between air-borne contamination and microbial population or keeping quality of the cheese. More information concerning the species identity of air-borne bacteria is desirable; however, the prevalence of yeasts and molds represents a hazard to quality. Many food plants have reduced air-borne contamination by ventilating with filtered, washed air and pressurizing processing rooms.

Yeasts and molds which tolerate low pH were the more comon contaminants in starter. When starters are ripened to an acidity of about 0.83 percent, the pH is sufficiently low to retard the development of most lipolytic, proteolytic and coliform bacteria; however, starters cannot be ignored as a source of these organisms, especially those of the lipolytic group, which includes the molds. Starter acidities above 0.85 percent should be avoided because of the reduced activity of *Streptococcus lactis*.

A few samples of coagulator were badly contaminated with lipolytic, proteolytic, yeast or mold organisms, with proteolytic organisms being the most frequent contaminant. These contaminants may be introduced by using non-sterile glassware in measuring coagulator and returning unused portions of coagulator to the original supply.

The number of organisms found in rinses from empty cartons picked up at cheese packaging stations indicates a need for improvement in storage and handling procedures. Cartons should be stored in a clean, dry place free from dust, moisture and air currents with particular attention to protection of opened cases.

The water supplies of many plants present a serious hazard to cottage cheese quality. Objectionable organisms may gain access to a water system in a number of ways including entrance through exposed open ends of water lines. *Pseudomonas* organisms are capable of moving through water lines and establishing themselves in the water system of a plant, because they are psychrophilic and are relatively non-fastidious with respect to nutrient requirements. High counts of undesirable organisms are frequently found in municipal and private water supplies which have been approved by public health inspection. There is an interesting relationship between the low coliform population and the significant lipolytic and proteolytic counts in the water samples examined in this work. Regardless of source, the dairy plant water supply should be examined frequently for all types of objectionable organisms and subjected to bactericidal treatment, if contaminated. Elliker (7) recommended the addition of 5 to 10 p.p.m. of chlorine to the water supplies of cottage cheese plants. Davis and Babel (5) reported that 8 of 15 slime producing organisms survived 1-minute exposure to 5 p.p.m. of chlorine. Marquardt (11) reported that 7.5 p.p.m. of available iodine added to water inhibited or retarded the organisms which commonly caused surface slime in cottage cheese. Collins (4) showed that pH and temperature are extremely important in the bactericidal treatment of dairy plant water supplies. The dairy plant water should be chlorinated close to the source in order to allow as much contact time as possible. Chlorination of the water at or near the vat is unsatisfactory. Davis and Babel (5) showed that chlorine is inactivated as soon as it comes in contact with the cheese. Extreme care must be exercised to avoid overchlorination because the cation of the chlorine compound may react with the curd to form a soft gelatinous surface. When the second wash water is refrigerated, the cooling equipment should be thoroughly sanitized. It is especially important that the washing process cool the curd as low as possible in order to minimize growth of spoilage and acid forming organisms during the interval prior to the time the cheese is placed under refrigeration. The introduction of insanitary water or steam hose, straining cloths, iron pipe, etc., into the vat during the cooking or washing operation should be avoided. A threaded sanitary cap should be used to cover the end of the water line when not in use.

The presence of spoilage organisms on vat surfaces and on various items of equipment was an important source of contamination. Sanitized equipment should be protected from splash and subjected to a chlorine solution of 100 p.p.m. immediately prior to use. Protection against condensate dripping from overhead pipes is necessary. Wooden equipment is virtually imposible to sanitize and should be discarded.

Without exception, swabs of wooden equipment were extremely high in count.

SUMMARY

Seventeen vats of cottage cheese were manufactured in 12 commercial plants. Microbiological analyses were performed on samples of all raw materials and of the cheese at various stages of manufacture. When held at 50° F., representative samples from 10 of the vats possessed shelf-life ranging from 5 to 10 days and the shelf-life of samples from 7 vats ranged from 13 to 16 days. In the fresh cheese the logarithmic averages of the counts of coliform, lipolytic and proteolytic bacteria were 50, 1,298 and 1,967 percent greater in the group of samples with the short shelf-life than in the group with the shelf-life of 13 to 16 days.

The initial organoleptic quality scores were only slightly higher in the group with the longer shelf-life. The pH of the cheese and temperature at which the product is washed and handled are important factors in keeping quality. The shelf-life of samples at 42° F. averaged 51 percent longer than corresponding samples held at 50° F.

Improperly pasteurized milk and cream, coagulator, starter, water, air and contaminated equipment were found to be sources of spoilage organisms. Samples of several of the above caused spoilage when inoculated into sterile cheese and incubated at 21° C. for 4 days. Several water suppleis were found which contained high populations of lipolytic and proteolytic organisms, but were free of coliform bacteria which are commonly used as an index of acceptability for water supplies. The varied sources of spoilage organisms indicate the need for observance of proper pasteurization procedures, equipment sanitation, precaution in handling raw materials and necessity for proper bactericidal treatment of wash water.

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