Microbial Studies on Shelf Life of Cabbage and Coleslaw

A. DOUGLAS KING, JR.,* H. DAVID MICHENER, HENRY G. BAYNE, AND KEIKO L. MIHARA

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Berkeley, California 94710

The microbiology of a common commercial type of coleslaw was investigated with the objective of extending its shelf life at refrigerator temperature by delaying microbiological spoilage. Cabbage, its principal ingredient, had a total bacterial count of about 10^5/g. Microbial growth in cabbage was prevented by storage at 1 C but not at 10 C or above. In coleslaw, the cabbage flora died and was replaced by the flora of the cultured sour cream contained in the dressing. At 14 C, the total count increased and the coleslaw deteriorated organoleptically. At 7 C, bacterial growth was suppressed but organoleptic deterioration occurred as rapidly as at 14 C. Thus, the deterioration was caused primarily by the physiological breakdown of plant tissue rather than by microorganisms, as was the original premise.

Manufacturers of delicatessen products desire a longer shelf life for salads and similar refrigerated foods. Coleslaw was selected arbitrarily as the first product for investigation. The coleslaw considered here is manufactured and packaged in central locations and distributed to stores and supermarkets for sale from refrigerated cabinets. The principal ingredients were cabbage and a dressing consisting principally of mayonnaise and cultured sour cream. To study its spoilage, we examined coleslaw ingredients and the finished product. Numerous minor ingredients were considered unlikely to contribute significantly to the microbial population, but they might influence the composition of the microbial flora. Coleslaw composition varies with the manufacturer, and other formulas include mayonnaise or salad dressing plus acidified sour cream. This investigation was begun with the thought that microbiological spoilage limits the shelf life of coleslaw.

MATERIALS AND METHODS

Cabbage was bought in local markets or produce houses and shredded in the laboratory with a knife or meat slicer to simulate commercial production of coleslaw. Coleslaw was bought in retail packages from local supermarkets. It was made by manufacturers who use cultured sour cream in the dressing. For laboratory-made coleslaw, cabbage was shredded, and the dressing supplied by the manufacturer of the coleslaw was added according to his specifications. In addition to other components, the commercial coleslaw and supplied dressing contained added sorbic, benzoic, lactic, citric, ascorbic, adipic and ethylenediaminetetraacetic acids. The pH of the coleslaw was about 4.2.

For total aerobic counts on cabbage and coleslaw, 50-g samples were placed in blender cups, diluted 10-fold with water, and blended for 2 min. Additional dilutions were made, using 0.1% peptone or Trypticase soy broth (BBL) as the diluent. Pour plates were made in duplicate in plate count agar (Difco) (1) and were incubated at 28 C for 2 days (cabbage) or at 20 C for 6 days (coleslaw). Enumeration of other microorganisms was accomplished with the following selective media: yeasts and molds, potato dextrose agar (Difco), pH 5.6 (1); lactic acid bacteria, liver sorbate agar, pH 5.5 (2). The genera of bacteria isolated from cabbage were identified by the scheme decribed by Harrigan and McCance (4) and Skerman (7).

Organoleptic tests of coleslaw were made by a triangle test, using twelve sets of judgments for each comparison or informally by two or three judges.

RESULTS AND DISCUSSION

Microbial population of cabbage. Over a period of 15 months, counts were made on chopped, market-fresh cabbage heads. The geometric mean of 33 samples was 2.3×10^5, with a 95% confidence interval of 9.3×10^3 to 5.6×10^6. These values are somewhat lower than the maximum counts previously reported (6).

The growing point of cabbage heads is inside at the tip of the cone so that the oldest leaves are on the outside. Most of the microbial population is on the outer leaves. The counts for bacteria from three heads averaged 1.4×10^6 and 3.8×10^2/g for the outside and inside (near the core) leaves, respectively. Counts of yeasts and molds followed a similar pattern. The outer leaves, in addition to having the highest microbial population, also are the darker-colored

leaves and contribute to the desirable green color of coleslaw.

Effect of temperature on microbiology of cabbage and coleslaw. Cabbage heads were stored at 1, 10, and 20 C, and periodically pairs were removed from each lot for counts. At 1 C the population showed little or no change for 6 weeks. At 10 C plate counts increased to over 10^7/g in 3 weeks, and the cabbage appeared spoiled at the end of that time (Table 1). At 20 C the counts increased almost as much in 8 days, after which time the cabbage was spoiled.

To investigate further the presence of psychrotrophs in cabbage heads, total plate counts were made on a lot that had been stored at 6 C for 4 weeks. Plates were incubated at 28 and 8 C, and the two temperatures gave about the same count (2.2×10^6 and 1.5×10^6, respectively). This and the increase in population at 10 and 20 C in the previous experiment indicate the presence of a large population of psychrotrophs. Thus, a mixed population grows on the cabbage heads. Literature reports indicate that the color and order stability of shredded cabbage is also temperature dependent (3, 5).

Because cabbage contains psychrotrophs and coleslaw is stored at a low temperature, it was considered probable that psychrophils would predominate in coleslaw. To count bacteria in coleslaw, plates were incubated at seven temperatures from 4 to 35 C. The highest counts were obtained at 20 C, and after 6 days of incubation no further increase was observed. There was not a sharp temperature optimum. All further counts were incubated at 20 C for 6 days.

Isolation of bacteria from fresh cabbage. In addition to the organisms shown in Table 1, colonies were isolated from fresh cabbage slurry after blending and plating. The 31 pure culture isolates belonged to the following genera: *Brevibacterium, Chromobacterium, Citro-*bacter, *Pseudomonas,* and *Xanthomonas.* Unfortunately, 11 isolates were lost before identification was complete, illustrating the fragility of some of these organisms when removed from their normal environment (6). These and related bacteria plus lactic acid bacteria are known to be normal flora on cabbage (6).

Microbial population on coleslaw during storage. Counts on 21 samples of commercial coleslaw varied between 5×10^4 and 5×10^6. The geometric mean was 1.8×10^5, with a 95% confidence interval of 4.8×10^3 to 7.0×10^6. During storage in the temperature range of 7 to 28 C, after a lag the count rose logarithmically at temperatures of 14 C and above, but at 7 C the count gradually decreased for 34 days (Fig. 1). A 10-fold or more increase in count was accompanied by a reduction in pH from 4.2 to about 3.3.

Source of bacteria in coleslaw. If bacteria introduced with the cabbage cause spoilage, the coleslaw made with outer cabbage leaves should spoil faster because of their higher bacterial population than that made with inner leaves. To test this hypothesis, two lots of coleslaw were made in the laboratory. The outer-leaf coleslaw contained cabbage from the outer 0.75 inch (ca. 1.9 cm) of the cabbage heads. The inner-leaf coleslaw contained the remainder of the heads, except for the cores. The finished coleslaw contained 25% of a 1:1 mixture of mayonnaise and cultured sour cream.

Total bacterial counts were made on the cabbage and on the mayonnaise-sour cream mixture (considered to be the only ingredients likely to have high counts). The difference between outer and inner cabbage leaves was less than indicated above, probably because of the use of larger portions of cabbage heads and

TABLE 1. *Effect of storage temperature on the microbial population of cabbage heads*

Storage	Plate counts on:	
	Plate count agar	Potato dextrose agar[a]
10 C		
0 Time	1.9×10^5	7.8×10^3
2 Weeks	4.2×10^6	4.4×10^5
3 Weeks[b]	8.2×10^7	2.6×10^7
20 C		
0 Time	2.0×10^5	6.4×10^4
8 Days[b]	1.1×10^7	5.5×10^6
16 Days[b]	3.8×10^8	2.2×10^8

[a] Primarily yeast.
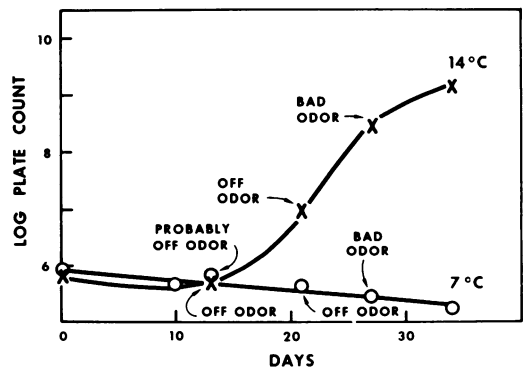[b] Spoiled (slimy, brown, bacterial rot).

FIG. 1. *Total aerobic bacterial count and odor judgment on coleslaw stored at 14 and 7 C. Above 14 C, growth was more rapid. At 7 C, the cold-susceptible organisms gradually died.*

differences between heads. Separate lots of outer- and inner-leaf coleslaw were made in which the mayonnaise-sour cream mix had been pasteurized (15 min, 60 C). These four coleslaw mixes were then incubated in sterile pint (ca. 0.473 liter) jars at 14 C and were sampled periodically for total counts (Table 2).

These counts show in two ways that the bacterial flora of the coleslaw during the first 15 days of storage was derived mainly from the sour cream mix and not from the cabbage. First, although the outer-leaf cabbage had over 10 times the total count of the inner-leaf cabbage, this difference was not strongly reflected in successive counts of the outer- and inner-leaf coleslaw. Second, when the mayonnaise-sour cream mix was pasteurized, the resulting coleslaw (whose flora was then presumably derived mainly from the cabbage) had a much lower total count than coleslaw containing raw sour cream. Furthermore, the count on the pasteurized sour cream coleslaw dropped rapidly during storage, showing that the cabbage-derived flora was dying.

The flora of the cultured sour cream was presumably lactic acid bacteria. Cabbage is also known to contain lactic acid bacteria (6). That the majority of the bacteria in the coleslaw was lactic acid bacteria was shown by nearly identical bacterial counts from coleslaw obtained on a medium selective for lactic acid bacteria, on liver sorbate agar (2.6×10^5/g), and on the plate count agar (2.2×10^5/g).

Nonmicrobial deterioration of coleslaw. Lots of outer- and inner-leaf coleslaw made with unpasteurized sour cream were stored at 3 and 14 C. If spoilage is of bacterial origin, the 14 C lots should deteriorate faster than the 3 C lots, because the low temperature suppresses

microbial growth. When these lots were tasted by the formal panel, the judges were never able to distinguish between the 3 and the 14 C coleslaw or the outer- and inner-leaf coleslaw. All samples were organoleptically unacceptable after 10 days. This shows that deterioration was not dependent on bacteria, because lots held at different temperatures and with different bacterial loads deteriorated simultaneously.

To investigate deterioration during a longer storage period, the 7 and 14 C storage samples described above (Fig. 1) were periodically compared organoleptically. Off odor (judged informally by three people) began to develop after 13 days at 14 C, before growth had progressed measurably. At 7 C, off odor was detected by one judge at that time. Both lots certainly displayed off odor at 20 and 27 days. Thus, they deteriorated almost simultaneously, although the bacterial population decreased slightly in one lot and increased logarithmically in the other.

Possibly, deterioration of coleslaw at 7 C was caused by microorganisms that we did not detect. If so, then fresh coleslaw should deteriorate more rapidly at this temperature if inoculated with coleslaw that had already deteriorated at 7 C. To determine whether this is so, fresh coleslaw was inoculated with 10% of the coleslaw that had been stored for a month at 7 C. With appropriate controls, as shown in Table 3, it was observed and smelled periodically to detect deterioration. The inoculated and uninoculated lots developed off odor at about the same time, showing that spoilage was not accelerated by the inoculum of deteriorated coleslaw or by bacteria that it contained. The plate counts did not increase.

The pH of coleslaw remained at about 4.2

TABLE 2. *Survival of microbial populations at 14 C in experimentally prepared coleslaw*

Determination	Total bacterial count/g				
	Initial	3 h	5 days	10 days	15 days
Ingredients[a]					
Outer-leaf cabbage	5.2×10^6				
Inner-leaf cabbage	4.3×10^5				
Sour cream-mayonnaise mix	3.3×10^5				
Coleslaw (unpasteurized sour cream mix)					
Outer leaf		6.9×10^5	4.2×10^5	3.1×10^5	2.4×10^5
Inner leaf		4.0×10^5	4.0×10^5	2.4×10^5	2.4×10^5
Coleslaw (pasteurized sour cream mix)					
Outer leaf		1.6×10^5	2.8×10^4	7.1×10^3	2.0×10^3
Inner leaf		1.2×10^5	7.0×10^3	8.7×10^2	7.0×10^2

[a] Microbial contribution of each ingredient to the completed coleslaw.

TABLE 3. *Effect of inoculation with deteriorated coleslaw on odor and microbial growth in fresh coleslaw at 7 C*

Stor-age time (days)	Odor[a]				Total bacteria counts/g		
	Fresh	Fresh + 10% spoiled	Fresh on day 14[b]	Deteriora-ted[c]	Fresh	Fresh + 10% spoiled	Deteriora-ted[c]
0	Fresh	Probably fresh		Spoiled	6.6×10^3	1.7×10^4	1.2×10^5
8	"Would eat"	"Would eat"		Spoiled	4.0×10^3	4.2×10^3	4.6×10^4
14	Sour, musty	Musty	Fresh		5.2×10^3	4.8×10^3	2×10^3

[a] Odor judged informally by three people.
[b] Freshly bought on day 14 of storage for comparison.
[c] Had been stored for a month at 7 C before this experiment began.

during deterioration at 7 C, but it dropped by about 1 unit during spoilage at 14 C, apparently as a result of microbial activity.

A microscopic examination may distinguish between a product spoiled by microorganisms and one not so spoiled. In this case, many bacteria could be seen by phase microscopy in the coleslaw deteriorated at 14 C, and only a few could be seen after deterioration at 7 C. (A phase-contrast microscope is more suitable than the usual bright-field microscope for finding bacteria among assorted food particles.)

Thus, deterioration at 7 C is not associated with increased plate count or the reduction in pH that is characteristic of microbial growth or microscopically visible proliferation of bacteria, and it cannot be accelerated by inoculation with previously deteriorated coleslaw. We conclude, therefore, that deterioration of coleslaw at 7 C (which is in the range of commercial storage) is not of microbial origin in the coleslaw that we investigated.

The cabbage portion of the coleslaw is living tissue that continues to respire and, with the storage time and conditions we have used in these experiments, it probably uses up the available oxygen. Shredded cabbage stored in a plastic bag for 2 days at 4.4 C reduced the oxygen content of the enclosed air to 10% and raised the carbon dioxide content to 12% (5). In coleslaw, the cabbage is subjected to further injury by being immersed in an oily emulsion (mayonnaise and sour cream), which probably impedes gas exchange between the cabbage and surrounding air. Lack of oxygen, resulting in tissue death and enzymatic degradation, is thus the probable main cause of deterioration.

LITERATURE CITED

1. **American Public Health Association.** 1966. Recommended methods for the microbiological examination of foods, 2nd ed. American Public Health Association, New York.
2. **Emard, L. O., and R. H. Vaughn.** 1952. Selectivity of sorbic acid media for the catalase negative lactic acid bacteria and clostridia. J. Bacteriol. 63:487–494.
3. **Francis, F. J.** 1960. Discoloration and quality maintenance in coleslaw. Am. Soc. Hortic. Sci. 75:449–455.
4. **Harrigan, W. F., and M. E. McCance.** 1966. Laboratory methods in microbiology. Academic Press Inc., New York.
5. **Kaufman, J., and J. M. Lutz.** 1954. Lengthening the shelf life of packaged coleslaw. Pre-Pack-Age 8:23–26.
6. **Pederson, C. S., and Margaret N. Albury.** 1969. The sauerkraut fermentation. Bulletin 824. New York State Agricultural Experiment Station, Geneva, N. Y.
7. **Skerman, V. D. B.** 1967. A guide for identification of the genera of bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.