DISTRIBUTION OF PROPIONIBACTERIA IN SWISS CHEESE

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SUMMARY

Conventional wheel and rectangular rind-bearing block Swiss cheeses were studied to determine the distribution of propionibacteria. Samples were taken from three predetermined locations in six different Swiss cheeses which were from 1 to 16 mo. old. Propionibacteria were determined by the plating method of Kambar *et al. (3)*, and cultures were taken from agar plates and identified. Few propionibacteria were found in samples taken 1/8-in. below the surface of the cheese. With one exception, samples taken 7/8-in. below the surface contained many more propionibacteria than were found in the exterior portions. Proceeding inward to sections 2⅝ in. below the cheese surface, the numbers increased, though not proportionally. These data suggest that the decrease in numbers of propionibacteria toward the exterior of Swiss cheese is probably the result of such unfavorable conditions as lower moisture, higher salt concentrations, and higher oxidation-reduction potentials.

From a bacteriological aspect, the outstanding characteristic of Swiss cheese is the presence of large numbers of propionic acid bacteria. The role of these organisms in the development of flavor in Swiss cheese has been clearly established by many workers. In numerous instances, the numbers of these organisms in cheese at various stages of the make and curing operation have been determined. No studies have been reported which have shown the distribution of propionibacteria within individual samples of Swiss cheese. The object of this investigation was to determine the influence of the usual make procedures upon the distribution of propionibacteria in Swiss cheese.

EXPERIMENTAL PROCEDURE

Cheeses. Information pertaining to the identification of the cheeses used in this study is shown (Table 1). Cheeses W1 through W4 were conventional wheel-shaped Swiss cheeses obtained through a commercial source. Cheeses B11 and B12 were experimental cheeses which differed from conventional Swiss cheese only in their shape; these were rectangular blocks, each weighing approximately 80 lb. Flavor and eye development in each cheese was considered good by a competent judge.

Sectioning cheese for analysis. A 4-in.-wide cross section was cut vertically through the middle of the cheese; from this section three layers parallel to the top surface were removed for analysis. The first section consisted of a layer 1/4-in. deep taken 1/8-in. under the surface of the cheese. This section hereafter is referred to as Position 1. Then, 1/2-in. of cheese was removed and discarded. A layer 1/4-in. deep was then taken; this section is referred to as Position 2. The next 1½ in. of cheese was removed and discarded. The next layer, 1/4-in.

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TABLE 1
Identification and description of experimental Swiss cheeses

Cheese No.	Form	Age
		(mo.)
B11	80-lb. Block	1
B12	80-lb. Block	1
W1	Wheel	2
W2	Wheel	2
W3	Wheel	16
W4	Wheel	16

deep, is referred to as Position 3. An 11-g. sample for plating purposes was taken from that portion of each layer that approximated the center of the cheese as viewed from the surface. Adjacent portions were taken for chemical analysis.

Bacteriological analysis. The cheese used for plating was weighed (11 g.) into a sterile container, then ground in a mortar to a smooth mass. During the grinding, 99 ml. of sterile aqueous 2.0% sodium citrate was added. Grinding was continued until a milky homogenous suspension resulted.

The plating method of Kambar *et al. (3)* was used. Duplicate plates were prepared. After counting, one plate which represented the proper dilution and possessed uniformly distributed bacterial colonies was selected for each cheese sample.

One hundred adjacent colonies from each plate were inoculated into tubes of the broth described by Kambar *et al. (3)* and were incubated at 30° C. After sufficient growth had occurred, the cultures were examined morphologically, using the gram stain. Cultures were tested for catalase production by combining 1 ml. of broth culture with 1 ml. of 3.0% hydrogen peroxide in a clean test tube. Cultures showing any evolution of gas were considered catalase positive. The ability of the cultures to ferment sodium lactate was determined. The medium used in these studies contained 0.5% Difco yeast extract, 2.0% B.B.L. Trypticase, 1.0% sodium lactate, and 1 ml. of a 1.6% solution of brom thymol blue. These ingredients were dissolved in distilled water; the broth was then adjusted to pH 7.0 with sodium hydroxide. Sterilization was accomplished by heating at 121° C. for 20 min. Cultures developing acidity in this medium were considered capable of fermenting sodium lactate. The appearance of the growth in broth developed by each colony was also recorded, as an aid toward classification.

Total aerobic counts were made, using the carrot-liver extract medium of Barber and Frazier *(2)*. This medium was modified slightly. B.B.L. Trypticase was used in place of Difco Neopeptone and the agar content was increased from 1.2 to 1.5%.

Chemical analysis. Standard methods *(1)* for the determination of moisture and salt were used. Samples for the determination of moisture were not preheated on a steam bath. Instead, samples were preheated in an oven without vacuum for 15 min. at 100° C.

RESULTS

The distribution of propionibacteria in Swiss cheese followed a definite pattern (Table 2). In all of the six cheeses examined, the propionibacteria de-

TABLE 2

Bacterial content and composition of six Swiss cheeses

Designation of cheese and sample location	Depth of sample from surface	Propionibacteria[a]	Total aerobic count[a]	Moisture content of cheese sample	Salt content of cheese sample	Salt in moisture
	(in.)	*(per g.)*			*(%)*	
B11–1	⅛	1	35	24.72	2.03	8.21
B11–2	⅞	700	186	36.60	1.27	3.47
B11–3	2⅝	1,350	246	38.77	0.31	0.80
B12–1	⅛	1	35	25.54	2.35	9.20
B12–2	⅞	1,200	151	35.74	1.29	3.61
B12–3	2⅝	2,170	189	38.19	0.23	0.60
W1–1	⅛	1	98	33.23	0.94	2.83
W1–2	⅞	74	216	37.72	0.80	2.12
W1–3	2⅝	390	202	38.73	0.36	0.93
W2–1	⅛	71	193	31.53	1.03	3.27
W2–2	⅞	1	38	37.48	0.95	2.53
W2–3	2⅝	410	228	38.87	0.42	1.08
W3–1	⅛	77	30	29.82	0.53	1.78
W3–2	⅞	440	43	36.05	0.65	1.80
W3–3	2⅝	610	21	37.38	0.69	1.85
W4–1	⅛	2	11	28.81	0.71	2.46
W4–2	⅞	17	33	35.91	0.89	2.48
W4–3	2⅝	107	19	38.39	0.86	2.24

[a] Counts expressed as multiples of one million.

creased in number from the interior portions of the cheese to the exterior. A minor exception to this pattern occurred upon examination of Cheese W2. Position 3 of Cheese W2 contained 410,000,000 propionibacteria per gram. Position 2, which was 1½ in. nearer the surface, contained less than 1,000,000 propionibacteria per gram. Position 1, however, located ½-in. nearer the surface than Position 2, and only ⅛-in. under the surface of the cheese, yielded 71,000,000 propionibacteria per gram.

Propionibacteria in Cheese B11 decreased from 1,350,000,000 per gram in Position 3 to 1,000,000 per gram in Position 1. Cheese B12, which was made on the same day and was cured simultaneously with Cheese B11, contained 2,170,000,000 propionibacteria per gram in the interior of the cheese (Position 3). Position 1 of this same cheese contained less than 1,000,000 propionibacteria per gram. Another example of this decrease in propionibacteria existed in Cheese W1. Position 3 in this instance contained 390,000,000 propionibacteria per gram; in Position 1, less than 1,000,000 per gram were present. Cheeses W3 and W4, which had been aged for 16 mo., again showed this typical distribution of propionibacteria. Cheese W3 contained 610,000,000 propionibacteria per gram at Position 3 in the interior of the cheese; Position 1 of this same cheese yielded 77,000,000 per gram. Cheese W4 at Position 3 had 107,000,000 propionibacteria per gram, whereas Position 1 yielded only 2,000,000 per gram.

As has been shown, propionibacteria are not distributed evenly throughout conventional block or wheel-shaped Swiss cheese. Samples taken 2⅝ in. below the surface of six different Swiss cheeses were, in general, quite high in numbers of propionibacteria. Proceeding outward, the number of propionibacteria decreased strikingly. All of the six samples taken ⅞-in. under the surface contained

fewer propionibacteria than were present in the sample areas closer to the center of the cheeses. The decrease in numbers of these organisms was most noticeable in the comparison between the samples taken 7/8- and 1/8-in. under the cheese surfaces.

Considering other types of bacteria, that is, those capable of growing aerobically on carrot-liver infusion agar, this pattern did not exist. Three of the six cheeses did not show any significant decrease in bacterial population of this type from the interior to the exterior of the cheese. In addition, organisms from the aerobic plates were cultured in litmus milk and were examined microscopically. There was no significant variation in numbers or types of these organisms throughout the six different cheeses. As would be expected, streptococci and lactobacilli predominated.

Results of the chemical analysis of the cheese for moisture and salt percentage were normal. In every instance, the interior portions of the cheese were higher in moisture content than the exterior portions. Considering salt content, the reverse of this was true, depending upon the age of the cheese. With cheese 2 mo. and younger, the salt was concentrated in the outer portions of the cheese. The salt in Samples W3 and W4, 16 mo. old, was distributed evenly throughout the cheese. When expressed as the per cent of salt in the moisture present at the different sample locations, this uniformity of salt distribution throughout Cheeses W3 and W4 becomes even more apparent.

DISCUSSION

The great variation in numbers of propionibacteria at the different sample locations is undoubtedly the result of several inseparable factors. Propionibacteria are characterized by their general inability to grow aerobically unless present in large numbers. During the earlier stages of Swiss cheese making, relatively few propionibacteria are present in the curd. It is during this time that the concentration of salt in the outer portions of the wheel is at its highest level. In addition, the outer portion of the curd mass at this time is low in moisture. High concentrations of salt in the moisture phase would inhibit growth of propionibacteria, especially if the oxidation-reduction potential were unfavorable.

At the same time, conditions at the surface of the cheese are at the most unfavorable, that is, during the early part of the warm-room treatment, conditions in the interior of the cheese would be most favorable for the growth of propionibacteria. Here, the curd would have a low oxidation-reduction potential, as has been determined in this laboratory. Moisture content would be comparatively high and salt content would be low. The temperature at this point is selected to favor the growth of the organisms (72–78° F.). Consequently, under these favorable conditions the propionibacteria in the interior of the cheese are able to increase greatly. At this same time, the same organisms in the exterior portions of the cheese are growing more slowly. This creates the great disparity in the specific bacterial population. After the warm-room treatment, the cheese is held at a temperature range (40–45° F.) not conducive to growth of propioni-

bacteria. As a result, the difference in numbers would have little chance to equilibrate itself.

REFERENCES

(1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. *Official Methods of Analysis.* Assoc. Offic. Agr. Chemists. 7th ed. Washington, D. C. 1950.

(2) BARBER, F. W., AND FRAZIER, W. C. Dissociants of Lactobacilli. *J. Bacteriol.*, **50**: 637. 1945.

(3) KAMBAR, C. S., REINBOLD, G. W., AND HUSSONG, R. V. A Plating Method for the Isolation and Enumeration of Propionibacteria. *J. Dairy Sci.*, **35**: 915. 1952.