1940

Defects of blue (Roquefort type) cheese

Higbee Wayne Bryant
Iowa State College

NOTE TO USERS

DEFECTS OF BLUE (ROQUEFORT TYPE) CHEESE

by

Higbee Wayne Bryant

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Dairy Bacteriology

Approved:

Signature was redacted for privacy.

In charge of Major work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
1940

TABLE OF CONTENTS

INTRODUCTION

Since the ripening of the different cheeses is primarily a biological process, variations in the results are to be expected. In blue cheese, as in most other types, some of the variations are of a minor nature and the cheese showing them are still considered satisfactory. In other cases the variations are of more importance, the cheese involved being definitely defective.

The objectionable conditions encountered in blue cheese vary widely, and some of them are difficult to classify. However, a number of rather specific defects have been noted often enough to make them of considerable practical importance. In general, the specific defects of blue cheese are essentially the same as those encountered in other cheeses.

An improvement in the general quality of blue cheese requires a reduction in the number of cheese showing minor variations from the most desirable qualities and the elimination of the cheese showing definitely objectionable conditions. As a basis for this, the causes of the various defects must be established.

STATEMENT OF PROBLEM

In the work herein reported, an attempt was made to determine the causes of a number of the more serious defects of blue cheese. The defects investigated are:

a. Soft edge defect of blue cheese

b. Gas formation in blue cheese

c. Lack of mold growth in blue cheese

d. Fruitiness in blue cheese

e. Black discoloration of blue cheese

f. Gray discoloration of blue cheese.

METHODS

Manufacture of Blue Cheese

All milk used for the manufacture of blue cheese was homogenized at 92°F. and a pressure of 2,200 pounds per square inch. Two per cent of a cheese culture was added to the milk and the milk ripened to an acidity of 0.19 to 0.20 per cent. Rennet was added at the rate of 3 ounces per 1,000 pounds of milk; it was diluted to 20 times its volume with water before addition to the milk. The milk was set at 88°F. A setting period of 1 hour was used, after which the curd was cut with one-half inch knives. The curd was allowed to set for 1 hour with occasional stirring and then dipped into swiss cheese cloths, where it drained. After draining for several minutes the mold powder was added and the curd hooped. The cheese were turned for the first time 15 to 20 minutes after hooping. They were turned again four or five times at increasing intervals during several hours and then drained over night. The cheese were dry salted at the rate of 6 pounds of salt (NaCl) per 100 pounds of green cheese. After salting the cheese were punched and placed in the curing room.

Manufacture of Cheddar Cheese

The milk used to make cheddar cheese was adjusted to 86°F. in a vat. Two per cent of cheese culture was added and the milk allowed to ripen to an acidity of 0.16 per cent, calculated as lactic acid. Cheese color was added at the rate of 1 ounce per 1,000 pounds of milk and rennet extract at the rate of 3 ounces per 1,000 pounds of milk. The rennet was diluted to 20 times its volume with water before being added to the milk. The milk was set for 25 minutes and then cut with three-sixteenth inch knives. The curd was cooked at 102°F. until the acidity in the whey reached 0.16 per cent and the desired firmness of the curd was obtained. After dipping, the curd was cheddared until about 0.5 per cent acidity in the whey was reached. Upon completion of the milling, the curd was forked for about 15 minutes and then 2 per cent salt was added. As soon as the salt had completely dissolved, the curd was placed in 5 pound hoops and pressed over night. The cheese were dried in a 50°F. refrigerator for 2 days and then paraffined. They were then placed in a 50°F. curing room to ripen.

Media Used

Preparation of tomato juice agar

Tomato juice agar was made as follows: For each liter, 400 ml. of tomato juice (obtained by filtering canned

tomatoes) was neutralized to a pH of 7.0. Ten gm. of Bacto peptone, 10 gm. of Bacto peptonized milk and 15 gm. of agar were dissolved in 600 ml. of water by boiling, after which the preparation was made up to the original weight and added to the tomato juice. The medium was autoclaved at 15 pounds pressure for 20 minutes, filtered and then distributed in tubes or bottles and again autoclaved.

Preparation of acidified tomato juice agar

Tomato juice agar was adjusted to a pH of 3.5 by adding a sterile 10 per cent solution of tartaric acid just before pouring into plates.

Preparation of Czapek's agar

Czapek's agar was made by dissolving the following ingredients in 500 ml. of distilled water:

Sodium nitrate	2.0 gm.
Mono-potassium phosphate	1.0 gm.
Potassium chloride	0.5 gm.
Magnesium sulfate	0.5 gm.
Ferrous sulfate	0.01 gm.
Sucrose	30.0 gm.
Agar shreds	20.0 gm.

After making up to 1 liter, the medium was autoclaved for 20 minutes at 15 pounds pressure. It was then filtered, distributed in tubes or bottles and again autoclaved.

Preparation of beef infusion agar

Beef infusion agar was prepared by the method outlined in the Committee Report on "The Microbiological Analysis of Butter" (7).

Plating Cheese

Preparation of sodium citrate solution

A 2 per cent solution of sodium citrate was prepared by dissolving a 2 gm. of sodium citrate in 100 ml. of distilled water. It was pipetted into test tubes in 9 ml. quantities, stoppered and sterilized in the autoclave. The tubes were stored in a refrigerator until used.

Preparation of fat emulsion

The fat emulsion was made by adding 0.5 gm. of agar and 3 ml. of cotton seed oil to 99 ml. of water in a screw cap bottle. The material was sterilized in an autoclave and allowed to cool. As the agar started to solidify, the bottle was shaken to emulsify the fat. The emulsion was stored in a refrigerator until used.

Preparation of plates

One gm. of cheese was weighed on a sterile piece of paper and transferred to a mortar. To this was added 9 ml. of a sterile aqueous sodium citrate solution. The cheese

was ground until a homogeneous suspension resulted. One ml. of the emulsified cheese was used in making subsequent dilutions. The dilutions employed in the plates were 1-10,000, 1-100,000, 1-1,000,000, 1-10,000,000, and 1-100,000,000. Beef infusion agar was used to make the total, proteolytic and lipolytic bacterial counts; for the proteolytic counts, 1 ml. of sterile skim milk was added to each plate before the agar was poured and for the lipolytic counts, 1 ml. of the fat emulsion was added.

Czapek's and acidified tomato juice agars were used in plating for molds, while tomato juice and acidified tomato juice agars were used for the detection of yeasts.

Determination of pH

Two gm. of cheese was placed in a mortar and ground to a thick paste. Ten ml. of boiled and cooled distilled water was added, and the mixture ground to a homogeneous suspension. Measurements were made with a potentiometer, using a quinhydrone electrode and saturated calomel cell.

Determination of moisture and salt (NaCl) in cheese

The methods adopted were those recommended by the Subcommittee Report on "Determination of Fat, Moisture, and Salt in Hard Cheese" (8).

Preparation of mold powder

Erlenmeyer flasks containing 0.5 inch cubes of sterile whole wheat bread were inoculated with a water suspension of mold spores. They were incubated at 50°F. until the bread cubes had become completely overgrown with mold. The contents of the flasks were then placed on cheese cloth and allowed to dry in a warm room. When dry, the bread cubes were ground in a mortar to a fine powder.

EXPERIMENTAL

Soft Edge Defect of Blue Cheese

A defect in which a portion of the edge of a cheese becomes soft is occasionally noted in blue cheese. The high humidity at which blue cheese must be ripened may favor the development of undesirable conditions other than those encountered in all ripened cheeses. The soft edge defect is of practical importance because the defective edge must be removed before the cheese is marketed.

General Observations

The defect in a soft edge cheese is confined to the edges and immediate vicinity. It usually penetrates to a depth of 0.25 to 1 inch and may extend completely around the cheese. If the cheese is not handled or disturbed, it appears normal in shape and color and has the usual slime formation on the surface. When the firmness of the cheese is tested by pressing with the thumb or fore finger, the top, sides and bottom of the cheese are firm while the edges are soft. There is no gradual softening as the defective edge is approached but rather a sharp dividing line between the firm cheese and the soft edge. The surface of these soft edges is very easily slipped off

exposing soft cheese beneath. The soft cheese has the color and consistency of a well ripened camembert. At first, the soft portion does not seem to have any off flavor or odor, but after a period of about 1 month it assumes a limburger-like character. This is confined to the soft material and appears to have little influence on the flavor of the normal portion.

Detailed observations on this defect were limited to one plant. The first signs of soft edge were noted on cheese which had been ripened from 4 to 6 weeks. In some of the racks of ripening cheese the defect had only developed on one or two end cheese. With others the defect had developed on the edges of one side of each of the cheese. In some instances the defect extended completely around each cheese, and when this occurred it was often found that the defect was much more pronounced on one side of the cheese than on the other; in some cases there was evidence of the defect on cheese in the adjacent rack. The defect was not present in all the cheese of a single day's make but frequently occurred in some of them and not in others. It was much more prevalent during the Spring and Summer than during the Fall and Winter.

Investigation showed that cheese in the curing room near the refrigeration pipes and the humidifier had the greatest tendency to develop soft edges. This would indicate that high humidity had something to do with the defect.

Other evidence to support this was the fact that if the position of the cheese in the curing room was changed, certain locations seemed to effect a partial recovery.

Experimental

Since the general observations indicated that excessive humidity in certain locations is closely related to the development of the soft edged defect in blue cheese, attempts were made to reproduce the defect under experimental conditions.

Trial 1.

Two small, 2.5 pound cheese, which were normal except for size, were placed in the curing room, one near the humidifier and the other as far as possible from any source of free moisture. After 1 month of ripening the cheese near the humidifier had developed a more luxuriant slime formation than the control cheese. The top, bottom, sides and edges of both cheese were normally firm. After 6 weeks ripening the cheese near the humidifier had developed soft edges, whereas the control had not, and after 2 months ripening the former cheese had developed an advanced stage of soft edge. The edges of the cheese had broken away in spots revealing smooth, creamy material having a limburger-like odor. In comparison the control cheese was firm and

appeared to be ripening normally. The cheese near the humidifier was moved to a position near the control and allowed to ripen another month. This resulted in a general firming of the outside of the cheese. At this time both cheese were cut and observed. The mold growth was good in both cheese. Neither cheese had developed more than a suggestion of a roquefort flavor. The body of the soft edge cheese was not as firm as the control, probably indicating a higher moisture content. A distinct division was noted between the soft edge and the normal cheese. The soft edge had penetrated to a depth of about 0.75 of an inch.

Trial 2.

After salting and punching three normal size cheese, a part of each cheese was covered with dry absorbent cotton. The cheese were then placed in different parts of the curing room to ripen. The positions chosen for the cheese represented the average ripening conditions in the curing room. The cheese were examined at 2 week intervals. The exposed portion of each cheese developed a normal slime formation, whereas the covered portion was slow in developing slime and the amount was less than average. The absorbent cotton remained dry for about a month, after which a gradual moistening took place. By the time the cheese had ripened for 2 months, those portions of the cheese covered with absorbent cotton had developed soft edge. With all three

cheese the soft edges had a tendency to penetrate deeper than usual. The defect was not confined entirely to the edges of the cheese but had a tendency to spread back from the edge and follow the outline of the absorbent cotton. The consistency of the soft edge material was characteristic of the defect, but it had not developed an off odor. When finally cut the normal portions of the cheese showed good mold growth and flavor development.

Trial 3.

During the making of two normal size cheese, a 2 inch cube from a peeled potato was placed in the curd used for one of them in such a position that it was near the center of the resulting cheese. After salting and punching, the cheese containing the potato (the experimental cheese) was partly covered with pliofilm while the other cheese (the control cheese) was not. In the curing room the cheese were placed near a humidifier to ripen. After a ripening period of 6 weeks, the unprotected portion of the experimental cheese and the control cheese had developed soft edge. The pliofilm was not removed from the experimental cheese but the cheese underneath appeared normal and was firm to the touch. After a ripening period of 2.5 months the unprotected portion of the experimental cheese and the control showed the soft edge defect in an advanced stage. The defect had spread back from the edge, and the soft

material had a limberger-like odor. The cheese under the pliofilm still appeared normal to the sight and touch. When cut, both cheese had a normal mold growth and a good flavor. The soft edge defect had penetrated about 1 inch. A sharp dividing line was noted between the normal cheese and the defective cheese. The half of the experimental cheese left unprotected appeared the same as the control cheese while the protected half was normal in all respects.

When the cheese containing the potato was cut, there was a cavity in it about the size of the original piece of potato. In this cavity a shrunken, brown, dry, mass was all that remained of the piece of potato. Evidently the cheese had dehydrated it. The results show how readily the cheese takes up moisture.

Trial 4.

After salting and punching three normal cheese, they were placed in a cooler to dry. Three days later the cheese were removed from the cooler and half of each was paraffined. The cheese were then placed near the humidifier in the curing room to ripen. At intervals the cheese were turned in the rack so that all parts of the cheese would be subjected to the same general conditions, especially exposure to free moisture. After ripening for 2 months the unparaffined portion of each cheese had developed soft edge while the paraffined portion was firm to the touch.

Two weeks later the cheese were cut. The unprotected half of each cheese showed a penetration of the soft edge defect of 0.25 to 0.75 inch. The mold growth was normal and the cheese had developed a good flavor. The cut surface of the protected portion of each cheese showed that there had been no softening under the paraffin. When the paraffin was removed, the surfaces of the cheese appeared to be just the same as they were the day they were paraffined. The mold growth in the portected parts of the three cheese varied. In two of the cheese the texture was fairly close and showed little mold growth; the flavor in this area was lacking. In the third cheese, however, the texture was more open and no difference in mold growth and flavor could be detected between the paraffined and the unparaffined portions of the cheese.

Trial 5.

A normal cheese was used to study the effect of certain hygroscopic materials with reference to their ability to produce the soft edged defect. After salting and punching, the cheese was placed on a board in the center of the curing room. Two aluminum rings, 0.5 inch deep and 2 inches in diameter, were used to keep the hygroscopic materials in definite areas. One ring was filled with calcium chloride and the other with sodium chloride. As these salts took up moisture and in turn were taken up by the cheese,

additional materials were placed in the rings. It was necessary to replace the calcium chloride much more often than the sodium chloride. This trial was continued for 2 months and although there was free moisture in the aluminum rings most of the time, the cheese failed to develop soft spots. Apparently, the hardening effect of the salts was greater than the softening effect of the moisture.

Comparative analyses of normal and defective cheese.

Five experimental cheese, which included a representative from each trial that had developed the soft edge defect, were analyzed to compare the normal cheese with the soft edge material. The comparison included moisture and salt contents, pH and counts of total, proteolytic and lipolytic bacteria. The data are presented in Table 1.

The moisture content of a normal cheese is known to vary in different parts. The general tendency is for the outer portions to be lowest in moisture. The analyses show that in every case there was a significantly higher moisture content in the defective soft edge than in normal portions of the same cheese, the differences varying from 8.19 to 16.91 per cent. The unusual relationship suggests that the outer portion of the cheese had taken up and retained considerable moisture.

Variations in salt content are noted in comparing different portions of the same cheese or different cheese

Table 1.

COMPARATIVE ANALYSES* OF NORMAL BLUE CHEESE AND MATERIAL FROM A SOFT EDGE

Cheese number	Per cent moisture		Per cent NaCl		pH		Bacteria, millions per gram					
							Normal cheese			Soft edge		
	normal cheese	soft edge	normal cheese	soft edge	normal cheese	soft edge	total	proteo-lytic	lipo-lytic	total	proteo-lytic	lipo-lytic
1	43.50	52.87	4.44	2.89	6.91	7.23	1,240	1.2	0.0**	3,616	4.1	0.0
2	35.16	52.07	3.70	3.16	6.09	7.32	106	0.0	0.0	1,910	0.0	0.0
3	45.21	53.40	4.68	3.28	5.46	7.23	5	0.0	0.0	128	0.0	0.0
4	39.37	51.32	3.85	2.56	6.00	6.84	61	0.0	0.0	1,380	0.1	0.0
5	37.58	50.98	3.38	2.49	6.13	6.78	9	0.0	0.0	880	0.0	0.0

* The values given are the averages of duplicates.
** No colonies on the dilutions poured.

from a lot. The variation in a cheese is greatest during the salting period, when the salt content of the outer portion is relatively high, and the content tends to become more uniform as the cheese ages because of the diffusion. In the analyses the normal cheese regularly had a higher salt content than the soft material, the differences ranging from 0.54 to 1.55 per cent. The lower salt content in the soft material presumably was the result of the water taken up by this material.

The data show that the soft edge material regularly had a higher pH than the normal cheese, the difference ranging from 0.32 to 1.77 pH units. There was considerable variation in pH between the different normal cheese and also between the different soft edges.

The numbers of bacteria in different parts of a cheese or different cheese normally vary. With a decrease in salt content, an increase in bacteria would be expected and this is clearly shown in the data. A direct microscopic examination not only confirmed the difference in numbers between the normal and soft cheese but also showed that the soft cheese contained a variety of bacteria, whereas the normal cheese contained largely cocci. The great variety of organisms in the soft cheese suggested the many morphologic types commonly found in the slime of normal cheese.

The softening of a cheese and the production of an offensive odor suggest a protein breakdown. If this were

due to bacteria, the cheese should show the presence of significant numbers of proteolytic types. The bacterial counts indicate that in no case were significant numbers of proteolytic or lipolytic bacteria detected in the soft material.

Figures 1. and 2. illustrate the soft edge defect in the cheese analyzed; figure 2. shows particularly the depth to which the softening may penetrate.

Discussion

The development of soft edge in blue cheese appears to be the result of an accumulation of moisture on the cheese. The logical place for softening to occur would be the edge, since here two surfaces are separated by a relatively small amount of cheese and the ratio of deposited moisture to cheese is high. Moisture deposited on other parts of the cheese would not have the same significance. The increase in moisture results in a decrease in salt content, and this in turn may permit greater activity of organisms or of their enzymes.

In a curing room a humidifier may be responsible for moisture being deposited directly on the cheese near it, the amount depending on the manner of operation and other factors. Ordinarily, a humidifier is essential in a curing room but if it is properly located and shielded

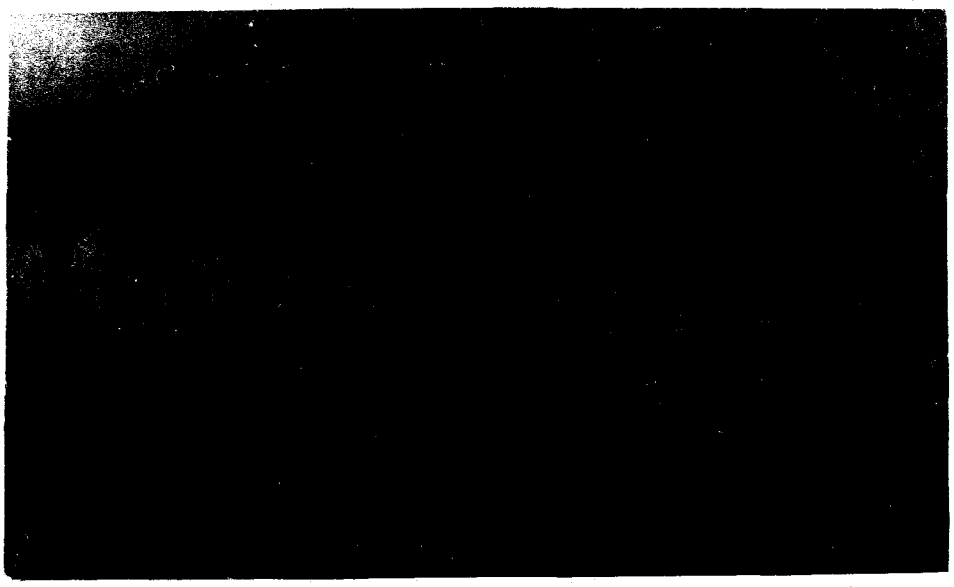

Figure 1. Cheese Showing Soft Edge Defect

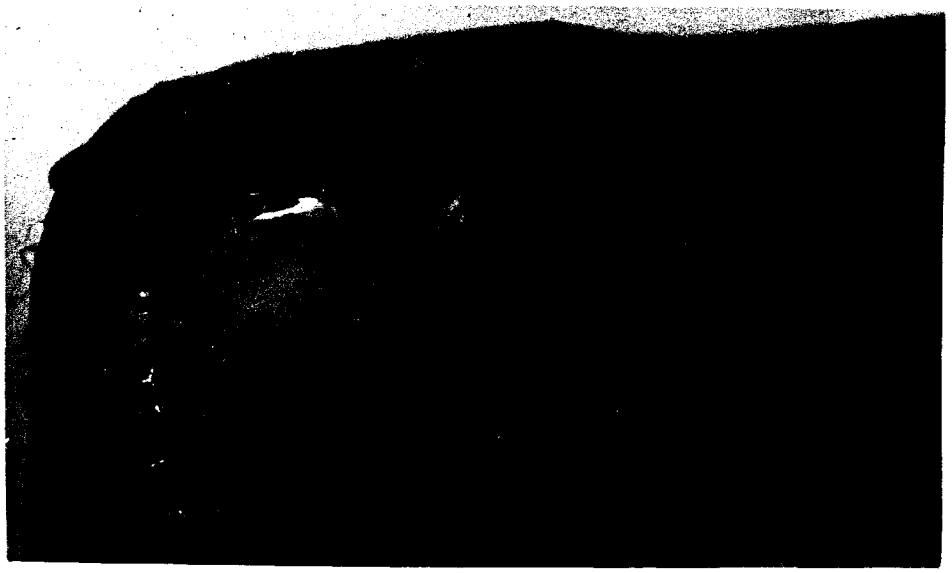

Figure 2. Cheese Showing Deep Penetration of Soft Edge Defect

there is little danger from it.

The refrigeration coils were also involved in the development of the defect in curing rooms. Variations in the brine often result in frost formation on the coils. When the coils warm and defrost, they evaporate moisture, some of which may be condensed on the cold cheese. If moisture drips from the coils it may fall on cheese or splash on it. Proper refrigeration readily eliminates the danger from the coils.

The reproduction of the soft edge defect by ripening cheese near a humidifier or refrigeration coils explains the defect in artificial curing rooms. While the defect appear to be unusual in caves, it has been noted there. Presumably, conditions in a cave could result in moisture being deposited on a cheese. If the air were saturated with moisture and fresh cheese were brought into the cave, air currents could carry moisture from the fresh cheese to the older cold cheese.

Conclusion

A defect of blue cheese in which a portion of the edges became soft appeared to be caused by excessive moisture in the softened parts of the cheese. The defect was readily reproduced by placing cheese close to a humidifier where free moisture could strike the cheese.

Gas Formation in Blue Cheese

Gas producing organisms are found in various dairy products, including cheese. In some of the swiss type cheeses, the characteristic eye formation is due to gas producing organisms, including those of the genus Propionibacterium, and is highly desirable. In other types of cheese, and particularly in cheddar cheese, the production of gas and the associated flavor constitutes a serious defect. The most common gas producing organisms causing defects in cheese are members of the Escherichia-Aerobacter group. Gas formation is known to occur in blue cheese but is of much less importance than in cheddar cheese.

General Observations

Gas formation has been observed in only a few blue cheese. The defective cheese were all imported, and while the volume of domestic cheese is small, no gassy cheese of this type has been observed. The defective cheese were not bulged and from the outside appearance did not disclose the presence of gas holes. The holes varied in size, were practically round and sometimes were present in moderate numbers. The mold growth was normal and the flavor did not seem to be affected.

Historical

The gassy defect in cheese has been studied by various investigators but, in general, they have confined their observations to cheese of the cheddar type. Moore and Ward (21) reported that a gassy condition in cheese curd, which was accompanied by an off odor, was due to an Escherichia-Aerobacter organism. Marshall (18) noted that the colon organisms produced gas holes in cheese alone but not when a good starter was added. In New Zealand, Whitehead (28) found that when colon types were added to milk, the resulting cheese developed unclean flavors but no gas holes. Leitch (17) reported that the production of gas in cheddar cheese curd was mostly due to the colon group but sometimes was due to Bacillus welchii or certain yeasts.

Experimental

Although the general observations indicate that gas production in blue cheese does not have the same significance that it does in cheddar cheese, several trials were carried out, to determine the importance of gas production by the Escherichia-Aerobacter bacteria in blue cheese, cheddar cheese being used as a control.

Trial 1.

A quantity of fair quality raw milk was obtained and divided into two equal portions of about 100 pounds each. The milk for blue cheese was homogenized, cooled to 60°F. and placed in a refrigerator. The milk for cheddar cheese was divided into two equal portions of approximately 50 pounds each, and each lot was placed in a compartment of a five section experimental cheese vat. Two per cent cheese starter was added to each portion. One portion was used as a control and to the other was added 10 ml. of a milk culture of Aerobacter aerogenes* which had recently been isolated from gassy cheddar cheese. The cheese was manufactured in the usual manner. When it had reached the cheddaring stage, the homogenized milk was taken from the cooler and the manufacture of the blue cheese was begun. This procedure was necessary because of the different temperatures employed in the manufacture of the two different types of cheese and because all five compartments of the vat were heated by the same jacket. The milk for the blue cheese was divided into two equal portions of approximately 50 pounds each and each lot placed in one of the unused compartments of the cheese vat. Two per cent cheese starter was added to each lot of milk. One

* The culture was identified on the basis of the characters listed in Bergey's Manual of Determinative Bacteriology (2).

compartment was kept as a control and to the other was added 10 ml. of the culture of A. aerogenes used with the cheddar cheese. The cheese was manufactured in the usual manner. The blue cheese curd from each compartment made one normal size cheese. The two cheese were salted, punched and placed in the curing room to ripen. The cheddar cheese curd from each compartment was pressed into a loaf of approximately 5 pounds. The two cheese were dried, paraffined, and placed in a 50°F. experimental cheese cooler to ripen.

After 1 month of ripening the experimental cheddar cheese was very slightly bulged and the cut surface showed large numbers of small gas holes. The flavor and odor were unclean and suggestive of gassy cheese. The control cheddar cheese had a normal outside appearance, and while the cut surface was solid for the most part, it had a small number of openings suggestive of gas holes. The flavor and odor, however, were mild and clean. The blue cheese were examined at this time also but were not cut. The outside appearance of each was normal, and several plugs drawn from each indicated that the mold growth and texture of both cheese were good, no off flavors or gas holes being noted.

After a ripening period of 2 months both the cheddar and the blue cheeses were cut. The observations made at the end of 1 month of ripening were confirmed.

Trial 2.

A good quality raw milk was obtained and trial 1 repeated, with the exception that to each experimental lot of milk 20 ml. of a milk culture of A. aerogenes was added instead of 10 ml. The cheese were allowed to ripen 2 months before being cut and observed. The experimental cheddar cheese was normal in shape but the cut surface showed large numbers of gas holes and the cheese had an unclean flavor. The control cheddar cheese was close textured, free from any openings suggestive of gas and had a mild, clean flavor. The experimental blue cheese showed a few small gas holes but the number and size were such that they would be noted only on careful examination; for the most part the holes were in the outer portion of the cheese. As is usual, the center of the cheese was more open than the outer portion. The mold growth in the cheese was normal and some of the characteristic flavor had developed; an unclean flavor was not detected. The control blue cheese was free of gas holes. The mold growth was normal and the cheese had begun to develop a desirable flavor.

Figures 3 and 4 show the cut surfaces of the experimental and control cheddar cheese.

Trial 3.

Milk similar to that used in trial 2 was pasteurized

Figure 3. Gassy Cheddar Cheese Made From Raw Milk Inoculated With Aerobacter aerogenes

Figure 4. Control Cheddar Cheese Made Without Inoculating Raw Milk With Aerobacter aerogenes

at 143°F. for 30 minutes, and trial 2 was repeated. The cheese were allowed to ripen for 2 months and were then cut and observed. The experimental cheddar cheese was not definitely bulged; however, it was filled with many small gas holes and had an unclean, slightly bitter flavor, with no typical cheddar cheese flavor. The control cheddar cheese was close textured and free of gas holes but was slightly bitter and lacked cheddar cheese flavor. Both blue cheese appeared normal and showed no gas holes; with each there was a more compact and brittle body than is normally found in raw milk cheese, and although there was good mold growth the cheese lacked flavor.

The cut surfaces of the experimental and control cheddar cheese are shown in Figures 5 and 6. From Figures 7 and 8 it is evident that the cut surfaces of the experimental and control blue cheese were essentially the same.

Trial 4.

Regular pasteurized market milk was used to repeat trial 3. The results were essentially the same as those obtained in trial 3.

Discussion

The much greater production of gas holes by A. aerogenes in cheddar cheese than in blue cheese presumably was due to

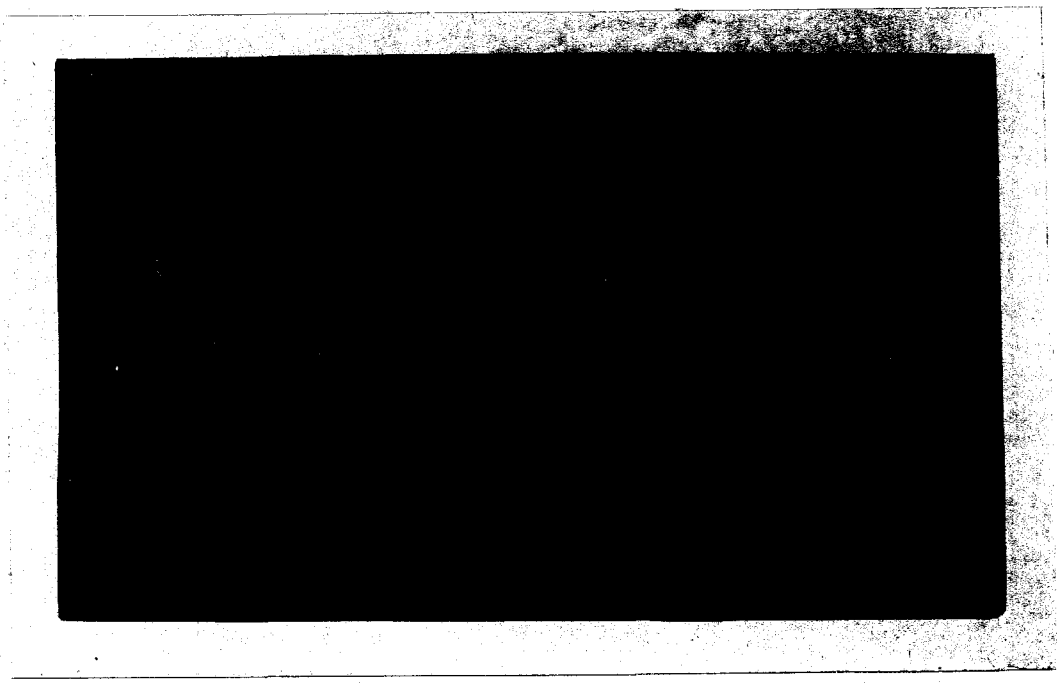

Figure 5. Gassy Cheddar Cheese Made From Pasteurized Milk Inoculated With *Aerobacter aerogenes*

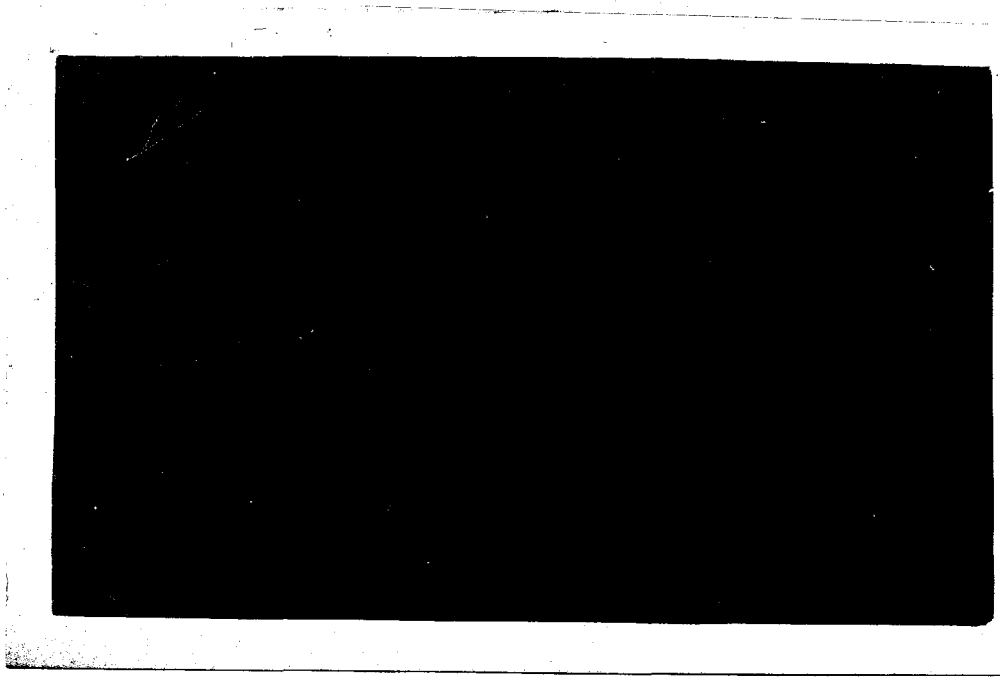

Figure 6. Control Cheddar Cheese Made Without Inoculating Pasteurized Milk With *Aerobacter aerogenes*

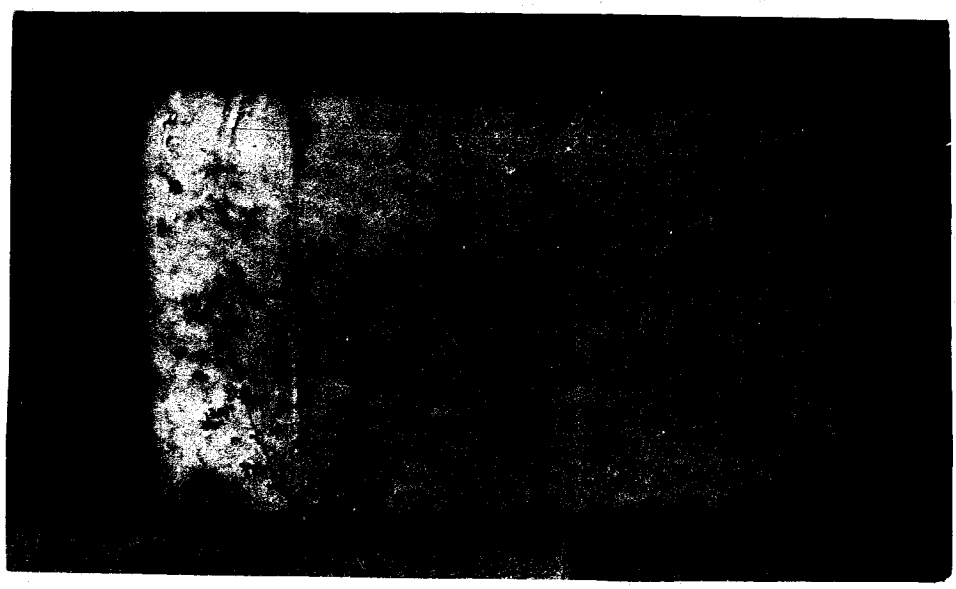

Figure 7. Blue Cheese Made From Pasteurized Milk Inoculated With Aerobacter aerogenes, No Gas Evident

Figure 8. Blue Cheese Made From Pasteurized Milk Not Inoculated With Aerobacter aerogenes, No Gas Evident

several factors. Perhaps the most important was the difference in texture between the two types of cheese. In cheddar cheese the texture is close and retards the escape of gas, while in blue cheese the texture is open and gas should escape readily.

In milk used for blue cheese, homogenization results in an early production of fatty acids which have an inhibitory effect on various bacteria. While the cheese culture organisms apparently develop rapidly in the milk, they are present in relatively large numbers and less effect would be expected on them than on species present in much smaller numbers.

The temperatures employed in making blue cheese are not as high, especially during the cooking process, as those used in cheddar cheese. The higher temperatures in cheddar cheese would tend to favor the development of A. aerogenes.

Presumably, the results obtained with A. aerogenes are essentially the same as those that would be obtained with other species of the Escherichia-Aerobacter group.

Conclusion

Gas formation is of relatively little importance in blue cheese, presumably because of the open texture, which permits the gas to escape, and the unfavorable conditions

in the cheese for the growth of the common gas forming organisms. Trials with a culture of A. aerogenes freshly isolated from gassy cheddar cheese showed that inoculations (of the milk) which resulted in very gassy cheddar cheese caused no gas holes or only insignificant numbers in blue cheese.

Lack of Mold Growth in Blue Cheese

A lack of mold growth occasionally is noted in blue cheese. In certain cases it probably is caused by too short a ripening period. This undoubtedly applies to some of the foreign cheese, especially when there is either a shipping deadline or a sharp increase in demand. With further ripening such cheese commonly develops a normal mold growth and flavor. In other cases the cheese lacks mold growth even after extended ripening. Often this defect is not discovered until after the cheese has been cut, at which time the cause is difficult to determine. Occasionally, however, the defect is discovered at such a time that definite information on it can be obtained.

General Observations

An opportunity to study an outbreak involving a lack of mold growth in blue cheese occurred when a commercial cheese plant experienced the defect. The outbreak occurred suddenly. A high quality cheese was produced up to and including the lot manufactured December 24, 1938. Cheese manufactured on and after December 25, 1938, did not develop normal mold growth or flavor. From the daily plant records it was noted that the cheesemaker had obtained from a laboratory a fresh supply of mold powder on December 24, 1938. This mold powder was used to make

cheese the following day and thereafter at the rate of about three times a week. The cheese were handled in the usual manner and, on the basis of slime formation, appeared to be ripening normally; the surface developed a good slime, and some cheese also had considerable mold growth on the surface. When the first few lots of cheese made with the new mold powder were about 2 months old, they were examined for mold growth by plugging two or three cheese in each lot. The plugs showed very little mold growth. However, since the outside appearance of the cheese was satisfactory, the cheesemaker concluded that the mold growth was slow and that it would develop later. The manufacture of cheese continued as scheduled. About 1 month later another routine mold examination showed the cheese lacking in mold growth. The cheese previously plugged were examined a second time and the plugs showed no improvement in mold growth. A systematic investigation of mold development was then begun. Several cheese in each lot were plugged and at least one cheese cut. Normal mold growth was not present in any of the cheese examined. Certain of the cut cheese showed areas of normal mold growth, while others were perfectly white. In some cases it appeared that the mold had invaded the cheese through the punch holes. From the observations the cheesemaker concluded that the lack of mold growth was due to defective mold powder and discontinued the manufacture of blue cheese until a new supply of powder could

be obtained. Upon receipt of the fresh mold powder the manufacture of blue cheese was continued. Several lots were made and kept under observation. The cheese were plugged several times, beginning at about 3 weeks and ending after 6 weeks of ripening. The presence of normal mold was not observed in any of the plugs.

The second apparent failure of the mold powder was called to the attention of the laboratory supplying the powder. The laboratory reported that another cheese plant had used a shipment of the same mold powder and had not encountered any difficulty in obtaining normal mold growth in its cheese. A new supply of mold powder was sent to replace that which was questionable. Cheese manufactured with this powder developed normal mold growth.

When the defective cheese were from 4 to 6 months old an effort was made to salvage some of them. The firmer cheese were repunched and inoculated with a water suspension of normal mold spores. This was successful in that many cheese later developed enough flavor to be salable. It was estimated that the outbreak involved some 5,000 pounds of cheese and that about 1,000 pounds were eventually marketed.

Experimental

A sample of the questionable mold powder was obtained from the commercial cheese plant. The powder was somewhat

lighter in color than normal. It was plated on Czapek's agar and the plates incubated at 21°C. Mold colonies developed rapidly on this medium and had a wide margin of sterile mycelium. The spores were blue green in color and spread from the center to the outside of the colony with age. While the colonies of the mold were not typical of Penicillium roqueforti, they were near enough to be accepted as one of the species of Penicillium used in making the blue veined cheeses. When the questionable mold powder was plated on acidified tomato juice agar under the same conditions, a very different type of colony developed. The colonies were more compact, slower growing, white in color and failed to produce a blue green color even after weeks of growth; they did not resemble colonies of the penicillia commonly used in blue veined cheeses. Flasks of whole wheat bread were inoculated with the questionable mold powder and incubated at 10°C. The resulting mold growth was blue green in color and appeared to be identical with that of normal P. roqueforti.

Trial 1.

An attempt was made to reproduce the defect in experimental cheese by inoculating with the original questionable mold powder. A quantity of blue cheese curd, sufficient to make three cheese of about 2.5 pounds each, was prepared. This was divided into three portions. To

one lot was added normal mold powder, to the second lot a mixture of questionable and normal mold powder and to the third lot only the questionable mold powder. The curd was then handled in the usual manner. After salting and punching the cheese, the surface of the cheese containing only the questionable powder was treated with calcium propionate and the cheese wrapped in parchment paper to limit mold contamination from the outside. The three cheese were then placed in the regular curing room. After a ripening period of 2 months the cheese were cut and observed. The cheese containing the mixed powder showed areas of normal mold growth and other areas in which no mold growth could be detected. The cheese containing the questionable powder did not show any mold growth. The control cheese had a normal mold growth. When the cheese had ripened for 3 months, they were cut and observed a second time. The cheese containing the mixed powder did not show any marked improvement in mold growth, although there was some increase. Flavor development was lacking. No mold growth or flavor was noted in the cheese containing the questionable powder. The control cheese had about the same mold growth as when first examined but was definitely lacking in flavor.

Trial 2.

Trial 2 was a repetition of trial 1 with the

exception that the cheese were of normal size and the one made with the questionable powder was not treated with calcium propionate or wrapped in parchment. The cheese were ripened 2 months and then cut and observed. The cheese containing the mixed powder showed a fair but rather irregular growth of normal mold but had not developed any appreciable flavor. The cheese containing the questionable powder showed an occasional small area of normal mold growth. These areas were near the surface of the cheese and appeared to have been due to an invasion of the cheese through the punch holes. The control cheese showed a good mold growth and had developed some flavor.

When the cheese had been ripened for 3 months, they were again cut and observed. The cheese containing the mixed powder had improved somewhat in mold growth and had developed a fair flavor. The cheese containing the questionable powder appeared about the same as at 2 months; however, in the small areas of normal mold growth some flavor development had taken place. The control cheese had about the same mold growth as at 2 months and had developed a fine flavor.

Trial 3.

Several samples of the original commercial cheese showing a lack of mold growth and several samples of experimental cheese showing the defect were plated in

the usual manner on Czapek's and acidified tomato juice agars. Characteristic colonies of the questionable mold were noted on plates poured with each cheese. Some of the colonies were picked and purified by repeated platings.

Powder A was prepared with a culture obtained from the commercial cheese by inoculating whole wheat bread in the usual manner. Powder B was made with a culture isolated from experimental cheese that lacked mold growth.

Sufficient blue cheese curd was prepared to make three normal size cheese. The curd was divided into three portions. To the first lot normal powder was added, to the second lot powder A was added and to the third lot powder B was added. The cheese were handled in the usual manner and after salting and punching were placed in the regular curing room. After a ripening period of 2 months the cheese were cut and observed. The two cheese made with powder A or powder B showed very little mold growth and had no flavor. The control cheese had a normal mold growth and had developed considerable flavor. After 3 months the cheese were again cut and observed. The cheese made with powder A and powder B were still lacking in mold growth and flavor. The control cheese appeared about the same as when previously examined.

Identification of mold in defective powder.

A number of mold cultures that had been isolated from

the original defective mold powder or from cheese made with it, as well as cultures from the experimental cheese that lacked mold growth, were studied in more or less detail. Identification studies were carried out with cultures grown on Czapek's agar. Microscopical observations and measurements suggested that the organism was Penicillium echinatum, according to the classification of Gilman and Abbott (11). Cultural characteristics suggested that the organism was P. roqueforti according to Biourge (3). The cultural characteristics were accepted rather than the microscopical observations and the mold is considered to be an atypical P. roqueforti.

Discussion

Several species of the genus Penicillium have been used successfully in the ripening of blue cheese but other species are not satisfactory. Although the organism present in the defective mold powder was regarded as an atypical strain of P. roqueforti, it did not develop well in blue cheese. The culture used to prepare the powder had been carried on an artificial medium for an extended period, and this may have resulted in a variation; however, there remains the possibility of the contamination of the culture with an organism which outgrew the original type.

Because of the very close relationship between

certain species of the genus Penicillium, it would be difficult to detect contamination or variation of a culture used to prepare mold powder. In case there was contamination, or variation the first indication might be the failure to produce normal mold growth in cheese.

The questionable mold powder was sent to two commercial cheese plants. One plant had difficulty in getting a normal mold growth with it while the other did not. The plant having difficulty received and used several pounds of the powder while the other plant received only 1 pound. At the plant having no difficulty, the cheese normally had an unusually extensive mold growth, although the rate of inoculation of powder was the same as in plants making cheese with less abundant mold growth. This indicates that the cheese made in the one plant was either receiving a natural inoculation of mold while being manufactured and ripened or that conditions were exceptionally favorable for mold growth in the cheese. The experimental results support the former assumption; cheese inoculated with questionable mold powder, treated with calcium propionate and wrapped in parchment paper failed to show any growth of normal mold, whereas cheese not treated with calcium propionate or wrapped in parchment showed some areas of normal mold growth. It appears that under certain

conditions enough natural inoculation occurs to produce fair mold growth and flavor in cheese.

In order to avoid slow mold development in the cheese, the mold powder must be carefully controlled. It is advisable to establish the effectiveness of a new lot of mold powder by using it in one or two runs of cheese before it is employed in more extensive operations. There may be an advantage in occasionally isolating a fresh culture of P. roqueforti from a good blue cheese and using it in the preparation of powder.

Conclusion

The lack of mold growth in the outbreak studied apparently was caused by the use of a mold powder in which an atypical strain of P. roqueforti predominated. The variation in the mold may have been caused by the long continued cultivation on an artificial medium of the culture used to prepare the powder.

Fruitiness in Blue Cheese

A sweet, fruit like flavor develops in cheese of various types. If the flavor does not develop in a cheese until after it is well ripened, it commonly is not conspicuous and is not severly criticized. Ocasionally, the flavor is noted in young cheese and then it is an indication that the cheese is not ripening properly. Fruitiness occurs rather frequently in domestic blue cheese. When slight, the defect is not objectionable to most people.

General Observations

In general, blue cheese with a fruity flavor have a normal appearance. The defect can be detected by smelling or tasting the freshly cut surface of the cheese. When the defect is slight, the fruity flavor seems to blend with the normal flavor of the cheese, but when it is pronounced the fruity flavor is conspicuous. In young cheese the defect is usually associated with a soft body, whereas in old cheese the defect is present in cheese with either a firm or a soft body. The presence of a yellow green mold has been noted in some fruity cheese, but such a mold has also been noted in cheese which were not fruity.

Historical

A fruity flavor is known to occur in various cheeses and is quite common in cheddar cheese. Harding, Rogers, and Smith (14) studied a "sweet" flavor defect in cheddar cheese and concluded that it was caused by yeasts. An outbreak of this defect in Canadian cheddar cheese was studied by Hood (15), who attributed it to the high yeast content of the cheese.

Experimental

Because of the apparent relationship between a soft body and high moisture content in cheese and the development of a fruity flavor, eight fruity cheese and eight normal cheese were analyzed for moisture and also for salt. Each cheese represents a different lot and all were obtained from one plant. The data are presented in Table 2.

From the results it is evident that the moisture contents of the fruity cheese were definitely higher than those of the normal cheese; the lowest moisture content of the fruity cheese was higher than the highest moisture content of the normal cheese. The average moisture contents of the normal cheese and the fruity cheese were 40.07 per cent and 46.25 per cent, respectively. Both the normal and fruity cheese showed wide variations in salt content,

Table 2.

MOISTURE AND SODIUM CHLORIDE CONTENTS OF NORMAL AND FRUITY BLUE CHEESE

Normal cheese		Fruity cheese	
Cheese number	Per cent moisture	Cheese number	Per cent moisture
1	41.66	1	49.76
2	42.03	2	47.23
3	34.71	3	46.74
4	35.62	4	45.32
5	39.31	5	46.52
6	39.44	6	46.96
7	44.72	7	45.36
8	43.09	8	50.17
Av.	40.07	Av.	46.25

Normal cheese		Fruity cheese	
Cheese number	Per cent NaCl	Cheese number	Per cent NaCl
1	4.96	1	4.36
2	4.30	2	4.51
3	4.36	3	5.52
4	5.32	4	4.83
5	3.90	5	4.08
6	4.46	6	4.15
7	4.58	7	4.60
8	3.95	8	4.78
Av.	4.48	Av.	4.60

but such variations are rather regularly noted in blue cheese from various sources. The average salt contents of the normal cheese and the fruity cheese differed very little, the values being 4.48 per cent and 4.60 per cent, respectively.

An attempt was made to duplicate the fruity flavor defect by making a cheese with a high moisture content. Sufficient cheese curd was prepared to make three normal size cheese. Curd for two cheese was dipped before it had firmed in an effort to increase the moisture content. It was inoculated with normal powder and handled in the usual manner. The remaining curd was allowed to firm normally and then dipped and inoculated with normal powder. After salting and punching the cheese were placed in the curing room.

When examined after 3 months the two experimental cheese had firm, compact bodies, and it was evident that the attempt to make cheese with a high moisture content was unsuccessful. The body was so firm that mold development was not satisfactory except in a few scattered areas. The cheese had a musty flavor, as is common when mold development in a blue cheese is slow and not abundant. The control cheese had good mold growth and flavor.

Since occasional samples of fruity cheese show the presence of a yellow green mold, attempts were made to isolate this microorganism so that its relationship to

the defect could be studied. Samples of fruity cheese were plated on tomato juice, acidified tomato juice, beef infusion and Czapek's agars. The plates were poured in duplicate and one set incubated at 10°C. and the other at 21°C. The plates were examined after 1 week and P. roqueforti was found on all plates. Microorganisms other than P. roqueforti occasionally were noted, especially colonies of a yeast and of a yellow green mold. These were picked to agar slants and litmus milk. The yeast did not ferment lactose and was not considered further. The yellow green mold produced a fruity, yeast like fermentation in litmus milk which suggested that it might cause a fruity flavor in cheese. A mold powder was prepared with it for use in experimental cheese.

Trial 1.

A quantity of blue cheese curd sufficient to make three 2.5 pound cheese was divided into three lots. To one lot was added normal mold powder. A mixture of normal and yellow mold powder was added to the second lot. To the third lot was added only yellow green mold powder. After salting and punching the cheese were placed in the curing room with other cheese made the same day. The three cheese were on the end of the rack next to the humidifier and the edges softened somewhat. The cheese nearest the humidifier was the control cheese and it

developed more of a soft edge than the others. After ripening for 2 months the cheese were cut and observed. The cheese made with the mixed powder had a good normal mold growth and the yellow green mold could not be detected; it lacked flavor. The cheese made with the yellow green mold powder had some normal mold growth but it showed very little flavor; the yellow green mold could not be detected. The control cheese had good mold growth but lacked flavor.

After a ripening period of 5 months the cheese were again cut and observed. Each cheese had a normal mold growth and the presence of the yellow green mold could not be detected. Both the experimental cheese and also the control cheese had developed a fruity flavor. This was more pronounced in the control cheese than in either of the experimental cheese to which the yellow green mold powder had been added. All three cheese had soft bodies, the body of the control cheese probably being the softest. The results suggest a relationship between a soft body in blue cheese and a fruity flavor.

Trial 2.

Sufficient curd was prepared to make three normal size cheese and the mold powders used in trial 1 were again tested. After ripening for 2 months the cheese were cut and observed. The cheese made with the mixed powder

showed a fair mold growth but no yellow green mold; no blue cheese flavor could be detected. The cheese made with only the yellow green mold powder had a few small areas of normal mold but no growth of the yellow green mold; the cheese had not developed a desirable flavor. The control cheese had a normal mold growth and a flavor characteristic of unripened blue cheese.

After 5 months ripening the cheese were again cut and observed. The bodies of the cheese were firmer than the corresponding small cheese. The normal mold growth had increased somewhat in the two experimental cheese, but the yellow green mold was not detected. The mold growth in the control cheese was essentially the same as at 2 months. Each cheese had developed some blue cheese flavor. It was most pronounced in the control cheese and least pronounced in the cheese made with the yellow powder. A fruity flavor was not detected in any of the cheese.

Additional attempts to isolate an organism causing fruitiness.

Several additional samples of fruity blue cheese were obtained. The cheese were approximately 2.5 months of age, had a good mold growth and had developed enough blue cheese flavor to be salable. The bodies of the cheese were softer than normal, and each cheese had a difinite fruity flavor. The samples were plated on Czapek's tomato juice, acidified tomato juice and beef infusion agars. The plates were

incubated at 10°, 21° and 37°C. They were examined each day for several days and very few microorganisms other than P. roqueforti were observed. Colonies of the yellow green mold previously isolated from fruity cheese were not detected.

Discussion

In the blue cheese studied, fruitiness most often occurred in cheese having a high moisture content, as shown by general observations on the body of the cheese and also by analyses.

The failure to isolate an organism capable of producing fruitiness in cheese suggests that the defect may be caused by a variation in the growth products of the normal blue cheese organisms, as a result of the change in environment. There remains the possibility of the isolation methods failing to yield the causative organism; most of the cheese examined were obtained after the ripening was complete.

Conclusion

A fruity flavor in blue cheese seemed to be associated with cheese having a relatively high moisture content. No organism capable of reproducing the defect could be

isolated from the defective cheese, and a yellow green mold present in some of the defective samples did not cause fruitiness in cheese, although it produced a fruity, yeast-like odor in milk. A relatively high moisture content in cheese may so influence the activity of the normal blue cheese organisms that the growth products deviate somewhat from the usual type.

Black Discoloration of Blue Cheese

Blue cheese is characterized not only by its flavor but also by the blue veins through it. The veins are due to the growth of P. roqueforti and are expected in a normal cheese. Molds other than P. roqueforti occasionally invade blue cheese. If the color produced by them is rather similar to that of the normal blue portions, it is overlooked, but if it is not similar, its presence is immediately noticed and it is designated as a color defect. One of the most noticeable of these color defects is a black discoloration.

General Observations

Samples of blue cheese having a black discoloration were received from a commercial cheese plant. The cheese showed little evidence of normal slime formation, but were dry at the surface and had cracked in several places. The surfaces of the cheese were darker in color than normal, and the cracks were filled with a black mold growth. Cut surfaces of the cheese showed that there had been an invasion of the cheese through the punch holes. They also showed a penetration of the black discoloration into parts of the cheese beyond the areas of black mold growth. There were many areas of normal mold growth and the flavor of the cheese was fair , although it tended to

be somewhat musty, especially in the discolored areas.

Historical

Microorganisms are known to cause various color defects in cheeses. Investigations have indicated that bacteria are responsible for most of these. Leitch (17) studied the cause of mottling and bleaching in cheddar cheese and concluded that bacteria were responsible and that colon forms accelerated discoloration. Gruber (13) isolated an organism causing small red or rust colored spots in North German hard and cream cheese. He named it Bacillus casei fusci. Thöni and Allomnn (26) attributed red spots in emmenthal cheese to a propionic acid organism, Bacillus acidi propionici var. ruber. Burri and Staub (4) described an organism responsible for red discoloration in emmenthal cheese and named it Bacterium subrufum. According to Davis and Mattick (10) Connell obtained the causative organism from cheddar cheese and named it Bacillus rudensis. Davis and Mattick (10) isolated the causative organism from cheddar cheese and concluded that it was related to the common lactic acid organisms. Stocker (24) found a mold of importance in producing color defects in cheese. He noted that black spots and dark discolorations on the rind of soft cheese were due to Monilia nigra. Discolor-

ation in New Zealand cheese was studied bacteriologically by Morgan (22); he noted that the muddy discolorations all occurred near cracks or openings in the cheese and concluded that they were caused by the growth of molds.

Experimental

Small portions of the defective cheese were plated, using Czapek's, tomato juice and acidified tomato juice agars. The plates were divided into three lots and incubated at 10°, 21° and 37°C, the incubation time being arbitrarily set at 1 week. An examination of the plates incubated at 37°C. showed very few molds and none suggestive of causing a black discoloration in cheese. Many of the plates incubated at 10° or 21°C. showed the presence of very dark mold colonies in addition to colonies of P. roqueforti. Of the three media, Czapek's agar appeared to be the most favorable for growth of the dark mold. Several colonies of the dark mold were picked to Czapek's agar slants and spotted on Czapek's agar plates for further study. Plating of the original cheese on Czapek's agar was repeated several times and in all cases plates incubated at 10° or 21°C. developed a significant number of very dark mold colonies.

Several samples of normal blue cheese were plated on Czapek's agar and the plates incubated at 10° and 21°C.

After 1 week the plates showed an occasional contaminating mold colony, but none were dark colored or in any way resembled the mold obtained from the defective cheese.

A preliminary examination indicated that all colonies of the dark mold were similar and were probably the same species. The fact that it was found in significant numbers in the defective cheese but could not be isolated from normal cheese led to the assumption that it was the mold responsible for the black discoloration. Mold powder was made with the dark mold, following the usual procedure, and several trials were carried out in an attempt to duplicate the defect.

Trial 1.

Sufficient blue cheese curd to make three 2.5 pound cheese was divided into three equal portions. To one lot normal P. roqueforti powder was added, in the second lot a mixture of P. roqueforti and the dark mold powder was used and to the third lot the dark mold powder was added. The lots of curd were then handled in the usual manner, and after salting and punching, the cheese were placed in the curing room. After 3 months the cheese were cut and observed. The outside of the cheese containing the dark mold was dark brown to black in color. The outside appearance of the control cheese and the cheese made with the mixed powders was normal. The cut surface of the

cheese containing the dark mold showed black mold growth in the punch holes, giving it a black streaked appearance. The cheese near the surface and next to the punch holes was discolored. A slight musty flavor had developed. The cut surface of the cheese made with the mixed powders showed a variation in the color of mold growth. Certain areas were blue, others dark to black and some appeared to be a mixture of the two. The black areas in the cheese were very small and the cheese had not become discolored outside the black areas. The cut surface of the control cheese showed only blue mold and was considered normal in color. Flavor development was not noted in either mixed mold cheese or the control.

Trial 2.

Trial 1 was repeated with the exception that the cheese were normal in size. After ripening period of 3 months the cheese were cut and observed. The outside appearance of the dark mold cheese differed from that of the corresponding small cheese. At first glance the surface of this cheese appeared quite normal, but when the surface slime and mold had been removed a large number of punch holes were very noticeable. They appeared as regularly spaced black pits in the surface of the cheese. The cheese in the immediate vicinity of these punch holes had begun to turn dark. The outside appearance of the

control cheese and of the cheese containing the mixed molds was normal and differed very little from that of the smaller cheese of trial 1. The cut surface of the dark mold cheese showed black streaks following the punch holes, and the cheese surrounding these streaks was dark. The dark mold had not spread through the cheese and was confined to the punch holes and openings leading from them. The flavor of the cheese was musty and did not in any way resemble blue cheese. The cut surface of the cheese containing the mixed mold powders showed normal blue areas, black areas and areas in which the mold appeared dark gray. The latter were probably caused by the two molds growing in close proximity. The flavor of the cheese suggested a combination of blue cheese and musty flavors. The cut surface of the control cheese was normal and the cheese had developed a fine flavor.

Trial 3.

This was a repetition of trial 2. Observations after 3 months confirmed those of trial 2.

Identification of dark mold.

Various cultures of the dark mold were studied in considerable detail. The organism was identified, according to the classification of Gilman and Abbott (11), as Hormodendrum olivaceum.

Discussion

Under natural conditions the invasion of blue cheese by H. olivaceum would be expected to occur through cracks and punch holes. If the texture of the cheese is open, the mold has a better opportunity to spread through the cheese and produce an extensive black discoloration. Under experimental conditions the growth of the mold was confined to the outside of the cheese and to punch holes. This indicates that H. olivaceum requires a good oxygen supply. From their investigations, Thom and Currie (25) concluded that the dominance of P. roqueforti in the interior of roquefort cheese is due partly to the reduced oxygen content which favors the growth of the normal mold over other types. For this reason H. olivaceum would not be expected to cause a great deal of spoilage in normal cheese. It is conceivable, however, that under certain manufacturing conditions, in which the cheese had a body particularly susceptible to cracking, the mold could cause extensive damage.

The musty flavor produced by H. olivaceum is of less importance than the discoloration. In experimental cheese made with a mixture of H. olivaceum and P. roqueforti, the flavor of the cheese was not typical but was close enough to normal to satisfy most consumers.

If the proper procedure is followed in making blue cheese, and particularly when homogenized milk is used,

the cheese will have a body that is not susceptible to cracking, and if the proper humidity is maintained in the curing room, the cheese will not dry and crack. Suitable manufacturing methods should do much toward controlling the defect.

Conclusion

A black discoloration and a musty flavor in commercial blue cheese were attributed to the growth of H. olivaceum in the cheese.

Gray Discoloration of Blue Cheese

Discolorations of cheese may be divided into two types, those involving color only and those involving flavor and color. Discolorations accompanied by a flavor defect are the more serious, and the gray discoloration of blue cheese belongs to this class. The defect is particularly serious because when once started it spreads through the entire cheese.

General Observations

Gray discoloration of blue cheese commonly is noted on the surface of the cheese after the slime has been removed by scraping or washing. The discolored portions are dark gray in color and vary from a few small areas on some cheese to complete discoloration on others. When defective cheese are cut they usually show a normal mold growth. The cut surface of different cheese show variations in the amount of discoloration. The defect appears to have originated on the surface and then to have spread through the cheese. Because of this the discolored portions evident on cutting the cheese vary from small areas to complete discoloration. In all

cases extensive discoloration is accompanied by a mousy, ammoniacal flavor which has a tendency to become soapy with age; the off flavor does not appear until the cheese are several months old. The defect seems to be confined to certain lots of cheese and may or may not include all the cheese in a lot. There appears to be no tendency for the defect to spread from one cheese to another.

Historical

Color defects in cheese have been observed and studied by numerous investigators. Dark discolorations, in which either bacteria or certain amino-acid were involved, have been noted in different cheeses.

Golding (12) investigated an outbreak of color defects in stilton cheese. The cheese first turned yellow but upon aging turned red and then black. Under practical conditions he found that the addition of large amounts of salt (NaCl) to the curd tended to favor the defect. Oxygen had a tendency to increase the darkening and from this he suggests that an oxidase might be responsible. This was further substantiated when heated cheese failed to turn yellow while chloroform did not stop the change. The addition of a solution of tyrosine to the cheese caused it to turn dark. From this Golding concluded that under certain conditions tyrosine may be a limiting factor.

Cornish and Williams (6), working on discolored stilton cheese, isolated many types of microorganisms and studied two groups. The first group, identified as Bacillus proteus vulgaris, produced a brown color both in solutions of typtophane and in tryptophane agar. In tyrosine media, however, it produced only a slight discoloration. The second group, composed of gram negative, alkali producing bacilli, gave a slight discoloration in tryptophane media and turned tyrosine media a dark brown or black. Further studies were carried out by Venn (27) and by Mattick and Williams (19). Venn used the gram negative, alkali producing bacilli isolated by Cornish and Williams and studied the effect of pH color on production in tyrosine media. He found that color was produced between pH 3.73 and pH 9.7, with the intensity increasing as the center of the range was approached. Mattick and Williams studied the Bacillus proteus vulgaris isolated by Cornish and Williams. They found that in typtophane solutions a color ranging from orange to light yellow was produced at pH values from 8.95 to 9.41.

Discoloration in New Zealand cheddar cheese was investigated biochemically by Moir (20) and bacteriologically by Morgan (22). Moir found that the pH of the muddy areas was much higher than that of normal areas in the same cheese. From this and other studies he concluded that the muddy discoloration is probably caused by

oxidative enzymes, including tyrosinase. Morgan noted that the muddy discoloration occurred near cracks or openings in the cheese and concluded that it was caused by the growth of mold.

The ability of certain microorganisms to produce black pigments has been studied. Skinner (23) found that nearly one-third of the Actinomyces he isolated were capable of producing a melanin when grown on Conn's complete medium plus tyrosine. Clark and Smith (5) reported that Bacillus niger produced a black pigment in protein media which contain metabolically available tyrosine.

A dark discoloration of cheese due to metals has been reported by a number of investigators. Hood and White (16) noted that light brown to yellowish brown areas in cheddar cheese were due to fragments of the steel wool which was used to clean the vat. Leitch (17) found that black discoloration in cheddar cheese was due to lead sulfide, while a gray black discoloration was due to iron sulfide formed under neutral or alkaline conditions. Davies (9) reported traces of tin in darkened areas of cheddar cheese but considered the lead constituent of the solder to be responsible for the color defect. Barnicoat (1) added various metals to cheese milk at the rate of from 3 to 7 parts per million. The discolorations noted with copper and iron were considered to be due to the atmospheric oxidation of a colorless metal protein complex, while the

discoloration with lead was due to its sulfides.

Experimental

Several investigators who have studied discolorations in cheeses have concluded that the color was due to melanins produced by the action of tyrosinase upon tyrosine. This suggests that perhaps there are microorganisms present in gray discolored blue cheese which are capable of producing melanins.

Blue cheese showing gray discoloration were obtained and cultured on several different media. Samples from the surface and the interior of the cheese were plated on Czapek's, tomato juice, acidified tomato juice and beef infusion agars. The plates were divided into three lots and incubated at 10°, 21° and 37°C. After the colonies were well developed, they showed a variety of colony types which included bacteria, yeasts and molds. The total number of microorganisms differed with each cheese plated. There was a variation in the flora of the different cheese, with P. roqueforti as the only microorganism which was consistently present on all plates. Representative colonies from each plate were picked into a medium consisting of skim milk saturated with l-tyrosine. The cultures were divided into two lots and incubated at 10° and 21°C. Observations were made each week on the cultures to see whether any of them were capable of darkening the medium. An occasional

culture produced a slight discoloration but discolorations were not considered significant and the cultures were discarded after an incubation period of 1 month. Additional samples of gray discolored cheese were plated, and similar results obtained.

The work of Skinner (23) suggests the possibility of Actinomyces being involved in the discoloration of blue cheese. Since blue cheese is usually made from raw milk, several samples of such milk were obtained and plated on beef infusion agar. The plates were divided and some incubated at 21°C. and the others at 37°C. The presence of Actinomyces was noted on plates from an occasional sample of milk. The colonies observed were of two types. One type produced a brown discoloration in the medium and the other did not. All colonies of Actinomyces were picked on beef infusion agar slopes and when a number of cultures had been cclleoted they were streaked on plates of beef infusion agar to which had been added 5 ml. of a skim milk tyrosine medium per plate. Tubes of the skim milk tyrosine medium were also inoculated with the organisms. Some of the cultures were incubated at 10°C. and others at 21°C. They were observed from time to time for color production. The cultures of Actinomyces which did not produce a discoloration in the beef infusion agar failed to produce a discoloration in the tyrosine media, and all cultures of Actinomyces producing a discoloration in beef

infusion agar produced a much darker discoloration in the tyrosine media. On plates the black discoloration extended beyond the limits of the colony. In the skim milk tyrosine medium the color ranged from a black at the surface to a dark gray at the bottom. This dark gray discoloration was similar to that found in a defective blue cheese.

Since the gray discoloration in cheese appears to originate at the surface and progress into the cheese, an attempt was made to reproduce the defect by inoculating pieces of cheese with the pigment producing Actinomyces. Portions from the surfaces of several normal cheese were placed in open mouthed glass containers and inoculated heavily with the Actinomyces. The containers were then held in a cooler, similar to a curing room, to determine whether the Actionomyces would grow and produce a gray discoloration in the cheese. After a period of 2 months no growth of Actinomyces could be detected, and as the cheese showed no signs of discoloration the samples were discarded.

The failure of the Actinomyces to grow and produce a gray discoloration in cheese suggests that conditions for growth in the cheese were not satisfactory. Since the organisms grew on agar and in milk with approximately the same pH as that found on the surface of a cheese, it would seem that from this standpoint cheese was a favorable medium. The high salt content of the cheese, however, might

be a factor in preventing the growth of Actinomyces. Several plates were prepared using beef infusion agar plus 5 ml. of the skim milk tyrosine medium to which had been added varying amounts of salt. The plates contained approximately 0.00, 0.01, 0.1, 2.0, 3.5 and 7.0 per cent salt. The plates were streaked with pigment producing Actinomyces, incubated at 21°C. and observed each day. The organisms grew on all the plates, and salt concentrations up to and including 2.0 per cent seemed to accelerate their growth. Higher concentrations definitely inhibited growth. In all cases the organisms produced a black discoloration and this seemed to vary in direct proportion to the amount of salt. The color production was greater in each plate containing salt than it was in the control.

The pH was determined on 13 samples of gray discolored cheese; in each case the pH was also determined on a normal colored portion of the same cheese. The data are presented in table 3.

The results show that a variation in pH occurred in both normal and discolored cheese. The pH range noted in the normal cheese was from 5.25 to 6.72 and in the discolored cheese from 6.25 to 7.29. The greatest variation in pH between normal and discolored portions of the same cheese occurred with cheese 11 and amounted to 2.04 pH units; the least variation occurred with cheese 2 and amounted to only 0.14 of a pH unit. The striking thing

Table 3.

pH DETERMINATIONS ON NORMAL AND GRAY DISCOLORED BLUE CHEESE

Cheese number	Normal cheese	Gray discolored cheese
1	6.07	6.38
2	6.11	6.25
3	6.15	7.12
4	6.16	7.18
5	6.09	7.12
6	6.72	7.02
7	6.50	7.03
8	6.15	6.85
9	6.32	6.83
10	6.00	6.65
11	5.25	7.29
12	5.56	7.22
13	5.77	7.28

shown by the data is that in every case the pH of the discolored cheese was higher than that of the corresponding normal cheese and in most cases the difference was significant.

Golding (12), in investigating an outbreak of color defect in stilton cheese, found that large amounts of salt favored the discoloration. Since stilton cheese is somewhat similar to blue cheese this suggests that perhaps the salt has something to do with the gray discoloration in blue cheese.

Six samples of defective and six samples of normal blue cheese were analyzed for salt. Each cheese represented a different lot and all were obtained from the same plant. Table 4 presents the results.

The data show a variation in the salt content of both normal and defective cheese. The average salt content of the normal cheese was 3.92 per cent while that of the defective cheese was 5.34 per cent. The significant thing shown by the analyses is that in all cases the salt content in the defective cheese was considerably higher than that of normal cheese.

Four cheese of average size were prepared to determine whether the gray discoloration could be produced by a higher salt content. The cheese were salted in the usual manner and were then placed in a saturated salt solution for 72 hours. The cheese were punched and placed

Table 4.

SODIUM CHLORIDE CONTENTS OF NORMAL AND GRAY DISCOLORED BLUE CHEESE

Cheese number	Per cent NaCl in	
	Normal cheese	Gray discolored cheese
1	4.30	5.05
2	3.60	4.55
3	3.95	6.06
4	4.08	5.99
5	3.70	5.10
6	3.90	5.32
Av.	3.92	5.34

in the curing room. After a ripening period of 3 months the cheese were cut and observed. The body of the cheese was firmer than usual and the mold growth was limited. The cheese had a fair flavor but the salt tended to mask it. The cheese was not discolored in any way and even appeared whiter than normal.

Discussion

The ripening of blue cheese involves a protein breakdown. Normally this does not proceed to a point where the resulting products affect the flavor of the cheese in an objectionable way. In cheese showing gray discoloration a mousy, ammoniacal odor commonly was present and the cheese tended to become soapy with age. The suggests a relatively large change in reaction, which is further indicated by the pH measurements on the cheese. Presumably, certain lower disintegration products of proteins which are basic in character are involved in the reaction change.

The variation in the normal ripening mechanism which yields cheese showing gray discoloration may be due to extensive growth of P. roqueforti since the organism actively attacks milk protein. Another possibility is that contaminating organisms are involved although none that reproduced the defect could be isolated. Certain Actinomyces

cultures produced a darkening in tyrosine media but they apparently failed to grow in cheese.

Conclusion

The development of gray discoloration in blue cheese was accompanied by an increase in pH. The variation from the normal ripening mechanism which caused the gray discoloration presumably involved the formation of basic products from protein. Extensive development of P. roqueforti or growth of contaminating organisms could be responsible for an unusual protein decomposition. Contaminating organisms capable of reproducing the defect could not be isolated.

RECAPITULATION OF CONCLUSIONS ON THE VARIOUS DEFECTS

A defect of blue cheese in which a portion of the edges became soft appeared to be caused by excessive moisture in the softened part of the cheese. The defect was readily reproduced by placing cheese near a humidifier where free moisture could strike it.

Gas formation is of relatively little importance in blue cheese, presumably because of the open texture, which permits the gas to escape, and the unfavorable conditions in the cheese for growth of the common gas forming organisms. Trials with a culture of Aerobacter aerogenes freshly isolated from gassy cheddar cheese showed that inoculations (of the milk) which resulted in very gassy cheddar cheese caused no gas holes or only insignificant numbers in blue cheese.

A defect of blue cheese in which the blue mold failed to develop in the cheese was apparently caused by the use of a mold powder in which an atypical strain of Penicillium roqueforti predominated. The variation in the mold may have been caused by the long continued cultivation on an artificial medium of the culture used to prepare the powder.

A fruity flavor in blue cheese seemed to be associated with a relatively high moisture content. No organism capable of reproducing the defect could be isolated from the defective cheese, and a yellow green mold present in

some of the defective samples did not cause fruitiness in cheese, although it produced a fruity, yeast like odor in milk. A relatively high moisture content in cheese may so influence the activity of the normal blue cheese organisms that the growth products deviate somewhat from the usual type.

A black discoloration and a musty flavor in commercial blue cheese were attributed to the growth of Hormodendrum olivaceum in the cheese.

A defect in which a gray discoloration and a mousy, ammoniacal flavor developed in blue cheese was accompanied by an increase in pH. The variation from the normal ripening mechanism which caused the gray discoloration presumably involved the formation of basic products from protein. Extensive development of Penicillium roqueforti or growth of contaminating organisms could be responsible for an unusual protein decomposition. Contaminating organisms capable of reproducing the defect could not be isolated.

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation for the assistance and suggestions given by Dr. B. W. Hammer in the experimental studies and preparation of this thesis; to Dr. M. P. Baker for his assistance in the preparation of the photographs; to Dr. J. C. Gilman for his assistance in identifying certain mold cultures; and to Mr. V. H. Nielsen for his cooperation in certain phases of the laboratory work.

LITERATURE CITED

1. Barnicoat, C. R. The effect of certain metallic contaminants on the cheddar cheese making process. Jour. Dairy Res. 8:53-60. 1937.

2. Bergey's Manual of Determinative Bacteriology 5th Edition. Williams and Wilkins Co. Baltimore, Md. 1939.

3. Biourge, Ph. Les moisissures du groupe Penicillium Link. La Cellule. 33:7-333. 1923.

4. Burri, R. and Staub, W. Eine eigenartige durch Bakerien bewirkte Rotfärbung in Emmentalerkäse. Landw. Jahrb. Schweiz. 40:1006-1011. 1926.

5. Clark, F. E. and Smith, N. R. Cultural requirements for the production of black pigments by bacilli. Jour. Bact. 37:277-284. 1939.

6. Cornish, E. V. C. and Williams, R. S. Colour changes produced by two groups of bacteria upon caseinogen and certain amino-acids. Biochem. Jour. 11:180-187. 1917.

7. Dairy Science Association. Committee on microbiological methods of examining butter. The microbiological analysis of butter. Jour. Dairy Sci. 16:289-299. 1933.

8. Dairy Science Association. Subcommittee for the analysis of cheese. Determination of fat, moisture, and salt in hard cheese. Jour. Dairy Sci. 20:27-30. 1937.

9. Davies, W. L. Detection in situ of tin solder causing dark discoloration in cheese. Analyst. 57:95-96. 1932.

10. Davis, J. G. and Mattick, A. T. R. Rusty spot in cheddar and other cheese. Jour. Dairy Res. 1:50-57. 1929.

11. Gilman, J. C. and Abbott, E. V. A summary of the soil fungi. Iowa State College Jour. of Sci. 1:225-345. 1927.

12. Golding, J. Yellow discoloration of stilton cheese. Jour. Bd. Agr. (England), 19:177-186. 1912.

13. Gruber, Th. Ueber die Ursache der braunroten Färbung von Hart-und Weichkäsen. Centbl. für Bakt. Abt. 2, 17:761-764. 1907.

14. Harding, H. A., Rogers, L. A., and Smith, G. A. Notes on some dairy troubles. N. Y. (Geneva), Agr. Exp. Sta. Bul. 183. 1900.

15. Hood, E. G. Some factors relating to injurious flavors in cheese. Butter and Cheese Jour. 20:18. December 25, 1930.

16. Hood, E. G. and White, A. H. A case of metallic discoloration of cheddar cheese. Sci. Agr. 10:520-522. 1930.

17. Leitch, R. H. Cheddar Cheese-Making. Faults in Cheese. The Scottish Agricultural Publishing Company Limited. 1932.

18. Marshall, C. E. Gassy curd and cheese. Mich. Agr. Exp. Sta. Bul. 183. 1900.

19. Mattick, E. C. V. and Williams, R. S. The influence of reaction on colour changes in tryptophan solutions. Biochem. Jour. 15:213-215. 1921.

20. Moir, G. M. Discoloration in New Zealand cheddar cheese. Muddy, bleached, and pink defects. Jour. Dairy Res. 4:238-245. 1933.

21. Moore, V. A. and Ward, A. R. An inquiry concerning the source of gas and taint producing bacteria in cheese curd. N. Y. (Cornell), Agr. Exp. Sta. Bul. 158. 1899.

22. Morgan, G. F. V. Discoloration in New Zealand cheddar cheese. Muddy, pink, and bleached defects. Jour. Dairy Res. 4:226-237. 1933.

23. Skinner, C. E. The "tyrosinase reaction" of the Actinomycetes. Jour. Bact. 35:415-424. 1938.

24. Stocker, W. Uber bakterielle Schwarzfärbungen des Käserinde. Centbl. für Bakt. Abt. 2. 82:405-406. 1930.

25. Thom, C. and Currie, J. N. The dominance of roquefort mold in cheese. Jour. Biol. Chem. 15:249-256. 1913.

26. Thoni, J. and Alleman, O. Ueber rote Punkte in Emmentalerkäsen hervorgerufen durch Bacterium acidi propionici var. ruber. Landw. Jahrb. Schweiz. 22:46-51. 1908.

27. Venn, E. V. C. The influence of reaction on colour changes in tyrosine solutions. Biochem. Jour. 14:99-102. 1920.

28. Whitehead, H. R. The influence of bacilli of the colon group on cheddar cheese. Jour. Dairy Res. 2:76-80. 1930.