The Role of Fungi in Cheese Ripening[1]

Fungi are important in the manufacture of two types of cheese—blue-veined cheeses, and Camembert and Brie. Among the former are Roquefort, Gorgonzola and Stilton, dependent on the mold Penicillium roqueforti and the bacterium Streptococcus lactis. Camembert and Brie require Penicillium camemberti and lactic acid-producing streptococci; the mold Oospora lactis and the organism Bacterium linens may also play roles in their manufacture.

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Introduction

Mold-ripened cheeses were manufactured and consumed in large quantities long before dairy microbiology acquired any importance. For many years the role of fungi in cheese-ripening was not clearly understood, and yet during this time cheeses of excellent quality were manufactured. Recent studies on the growth and chemical activities of fungi in cheese-ripening have resulted in the adoption of scientific procedures for cheese manufacture which permit a large proportion of the products to be uniform in flavor.

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Fungi are important in the manufacture and ripening of two types of cheese, namely, the blue-veined cheeses and Camembert cheese. Both types had their origin in France. The blue-veined cheeses, as their name implies, are those in which blue-green mold can be seen growing throughout their interior. Common among the blue-veined varieties are Roquefort cheese manufactured from sheep's milk and originating in France, Gorgonzola made from cow's milk and originating in Italy, Stilton cheese manufactured from cow's milk and originating in England, and various types of Blue cheese or Roquefort-type cheese manufactured from cow's milk in the United States and many other countries. Camembert is characterized by a white mold growth on the surface and a soft texture when properly aged; it is about one inch thick and four inches in diameter. Cheese manufactured by the Camembert procedure and identical to Camembert except for their larger diameter are Brie cheese. Both Camembert and Brie are made from cow's milk.

Blue Cheeses

Early History in the United States. Prior to 1900 various individuals and cheese companies attempted to manufacture blue-veined cheese from cow's milk in the United States with the idea

of duplicating the Roquefort cheese imported from France. None of the attempts was successful. In 1903, Professor H. W. Conn of the Storrs Agricultural Experiment Station, Storrs, Conn., began a systematic microbiological study of blue-veined and Camembert cheese (3). Molds were isolated from many imported cheeses and their physiology studied. Later, investigations were conducted jointly by the Storrs Experiment Station and the United States Department of Agriculture. Some success in the manufacture of a Roquefort-type cheese was attained and methods for manufacture were published (19). However, large-scale commercial production did not result. Renewed interest in blue-veined cheese was evident after World War I, and in 1921 a bulletin on the manufacture of cow's milk Roquefort was published by Matheson of the United States Department of Agriculture (14). One of the criticisms of the domestic cheese concerned its yellow color, especially when milk was obtained from cows on pasture.

In the early 1920's an appreciable amount of goat milk was available in California, and this milk appeared suitable for blue-veined cheese. Cooperation between the United States Department of Agriculture and the California Agricultural Experiment Station resulted in a series of investigations and the publication of a method for the manufacture of Roquefort-type cheese from goat's milk (8). The cheese thus manufactured was whiter than cow's milk cheese and more similar to Roquefort. Later, Goss et al. (7) of the Iowa Agricultural Experiment Station published a procedure for the manufacture of Iowa Blue cheese.

Several commercial organizations attempted to manufacture blue-veined cheese of the Roquefort type by the various methods that were published, but, because of several difficulties, no large industry resulted. The difficulties encountered included failure of the mold to develop properly, yellow-colored cheese, no development of the characteristic flavor in a reasonable length of time and lack of uniformity among lots. Also, the cost of manufacture of the domestic product was greater than that of the imported.

Some of the early experimental work on the flavor of Roquefort was conducted by Currie (4) who pointed out that the flavor is partly due to the accumulation of caproic, caprylic and capric acids during ripening.

Lane and Hammer (12) of the Iowa Agricultural Experiment Station concluded that if fatty acids were responsible for the flavor of Blue cheese, perhaps the flavor could be produced more quickly if the milk were homogenized before being made into cheese. The homogenization process greatly decreases the size of fat globules in milk and therefore increases the fat surface. Raw milk develops a rancid flavor characterized by free fatty acids very quickly after homogenization due to the presence of milk lipase and the increased fat surface.

A preliminary report on the manufacture of Blue cheese from homogenized cow's milk was published in 1936 (12). These experiments indicated that the curd obtained from homogenized milk was more flaky and had less yellow color than cheese prepared from nonhomogenized milk. Another important characteristic of the homogenized milk cheese was that mold growth was more luxuriant. The cheese manufactured from homogenized milk developed a definite rancid flavor early in the ripening period, and there was evidence of free butyric acid. Later in the ripening period, the cheese made from homogenized milk were no longer rancid, nor was there evidence of butyric acid. The disappearance of rancidity and butyric acid was accompanied by the development of a

sharp peppery flavor characteristic of Roquefort cheese.

The method of manufacturing Blue cheese by use of homogenized milk (13) was quickly accepted by the cheese industry. Individuals and cheese plant operators began a search for suitable locations for factories which had natural ripening facilities. An abandoned brewery along a river at Faribault, Minn., was chosen as the site for one cheese factory because a cave in the bank at the side of the plant appeared to have ideal temperature and humidity conditions for blue-veined cheese. The French people who settled at Nauvoo, Illinois, many years ago used caves to age wine, and these caves provided a site for another Blue cheese factory. Another series of caves were located near the Mississippi River at St. Paul, Minn., and cheese was transported to those caves for curing. Abandoned coal mines and mushroom cellars were converted to ripening rooms. Besides these natural ripening facilities, many curing rooms were constructed and fitted with temperature and humidity controls. An extensive Blue cheese industry was operating in the United States prior to 1939 or within three years after the homogenization process was developed.

Present Manufacturing Procedure. The manufacturing procedure used by a large number of Blue cheese manufacturers in the United States today is as follows:

Whole milk containing approximately 3.5 percent butterfat is warmed to 85° F and separated. The skimmilk is directed into the cheese vat, and the cream is run through a homogenizer operating at 2500 pounds pressure per square inch. The cream from the homogenizer is directed to the cheese vat and mixed with the skimmilk. During the season when cows are on pasture, it is common practice to bleach the fat of milk with benzoyl peroxide so that cheese having a uniform color throughout the year can be manufactured. One percent of an active culture of lactic acid-producing streptococci is added to the milk in the cheese vat. The milk in the vat is adjusted to 90° F and allowed to stand for about one hour in order to develop a small amount of lactic acid. The period allowed for growth and acid production is known in the cheese industry as the ripening period. After the ripening period, a solution of rennet is added to the milk at the rate of three or three and one-half ounces per 1000 pounds of milk. Rennet is an extract prepared from calves' stomachs which contains a definite quantity of the enzyme rennin. In the manufacture of Blue cheese, the milk shows signs of coagulation 12 or 15 minutes after the addition of rennet. However, the rennet is allowed to act on the cheese milk for one hour so as to form a firm coagulum and to permit more acid production by the lactic culture. The time during which the rennet is permitted to act is known as the setting period. After the setting period, the vat contents appears as a firm smooth coagulum. The coagulum is then cut with curd knives into cubes about one-half inch square. After cutting, the curd particles are not disturbed for about 15 minutes to permit them to firm slightly and to allow whey to be expelled from the particles. Throughout the entire manufacturing procedure the vat contents are maintained at 90° F. About 15 minutes after cutting, the contents of the cheese vat are stirred slowly. Stirring assists in the removal of whey from the curd particles and aids in firming the curd. The stirring is intermittent and generally carried out for one to one and one-half hours. The time of stirring is dependent upon the rate of acid formation and the rate of firming of the curd. It is desirable to produce a firm curd with as low acid production as possible. When the curd particles seem firm, all

or a portion of the whey is drained from the vat. Some manufacturers prefer to dip the curd out of the whey onto a draining rack in order to prevent the curd particles from matting, while others prefer to drain all of the whey from the vat. The latter method is more time-saving but may result in considerable matting if not carried out rapidly. Excessive matting is undesirable because mold powder cannot be mixed evenly with the curd, excessive fat loss may take place if the matted curd is broken, and the curd may have too close a texture (curd particles too closely knitted together). After the curd is drained free of whey it is mixed with one percent salt and approximately 0.01 percent of mold powder (*Penicillium roqueforti*). The curd is placed in perforated forms about seven inches in diameter and eight inches high with open ends. The forms or curd hoops are placed on a table covered with open-textured cloth, wire mats or perforated metal sheets. The curd quickly mats and assumes the shape of the hoop. The cheese are turned in the hoops each half hour for two hours, each hour for the next six hours, and once every two hours for the next eight hours. By this time the cheese are firm and can be removed from the hoops to the salting room. The salting room is maintained at 50° to 55° F and 95 percent relative humidity. The cheese are salted by applying dry salt to the surface each 24-hour period until the cheese contain about four percent salt.

After the cheese are salted, they are pierced (skewered) on the flat surface with wire needles so that a punch hole occurs in each three-quarter square inch area. When punched, the cheese are ready for the curing room. Curing or ripening is accomplished by holding the cheese at a temperature of 50° to 55° F and 95 percent relative humidity for about three months. Before the cheese are marketed, they are washed and wrapped in tin foil.

Preparation of Mold Powder. During the early period of Blue cheese manufacture, mold powder was prepared by growing a strain of mold in the interior of a loaf of bread. Fresh bread or older bread was sterilized with dry heat (170° C for two hours) and then permitted to cool to room temperature. Mold spores obtained from an agar slant were suspended in sterile water and added to the bread by piercing the surface with a pointed pipette and allowing the spores to flow into the loaf. The inoculated bread was held in a moist cool place for two or three weeks to permit the mold to develop, and then removed to a dry room. When the bread was thoroughly dry, it was sliced and ground to a fine powder with mortar and pestle or by use of a mechanical grinder.

It was noted (11) that culturing mold in the above manner frequently resulted in the development of foreign molds. Also, powder with a low spore count often was obtained because of failure of mold to grow in portions of the loaf. The long time required for the mold to develop was objectionable also. The investigators compared several procedures for preparing mold powder and developed the method which is in general use at the present time. This method consists of cutting whole wheat bread into half-inch cubes. The bread is placed in Fernbach flasks, filling them about one-third full. After stoppering the flasks with gauze-covered cotton, they are sterilized by autoclaving at 15 pounds pressure for one hour. Following the heating, the flasks are shaken repeatedly during cooling to keep the bread cubes

FIG. 1 (*Upper*). Cutting curd in the manufacture of blue cheese.

FIG. 2 (*Lower*). Blue cheese being turned to facilitate whey drainage and to form the cheese. (*Photos by courtesy of Maytag Farms, Inc., Newton, Iowa.*)

from sticking together. When cool, the flasks are inoculated with a suspension of mold spores. At this time a small quantity of sterile water may be added to provide sufficient moisture for rapid mold growth. The flasks containing bread are incubated at 70° F for eight to 12 days and shaken daily during the incubation period. Appearance of mold growth within the flasks determines the incubation period. When the bread cubes attain a blue-green color the flasks are emptied onto drying trays which are placed in an oven maintained at 110–120° F. After drying, the moldy bread cubes are ground to such fineness that the powder will pass through a 40-mesh screen. The ground mold powder is placed in tin cans, sealed and held in a cool place. Mold powder prepared in this manner will have a spore count of 500 million to five billion per gram. Generally, five or ten grams of powder is sufficient for the inoculation of 100 pounds of cheese curd.

The Role of Microorganisms. Although several types of microorganisms may be present in blue-veined cheese, only two are essential for manufacture, ripening and flavor development. These organisms are *Streptococcus lactis* and *Penicillium roqueforti.*

Streptococcus lactis is a bacterium which is always present in raw milk and is characterized by its ability to ferment lactose rapidly with the production of lactic acid. This bacterium is of considerable importance during the manufacturing process. A small amount of lactic acid is beneficial from the standpoint of coagulation of milk with rennet. The rennin enzyme present in rennet coagulates milk in a short time in the presence of a slight quantity of acid. Growth of and acid production by *S. lactis* during the manufacturing procedure aid in removing moisture from the curd particles and in firming the curd. Also, by lowering the pH of the cheese a medium is provided which is unfavorable for the growth of many other bacteria. The lactic acid-producing streptococci complete their role in a short time, and analyses indicate that viable organisms of this group have largely disappeared from the cheese shortly after salting or after about ten days.

Penicillium roqueforti is a mold which produces both proteolytic and lipolytic enzymes. Also, the mold is capable of growing in a low oxygen tension and in the presence of considerable sodium chloride. These characteristics make the mold ideally suited for Roquefort-type cheese. The action of *P. roqueforti* may be observed soon after the cheese are manufactured. Frequently, the day following manufacture a slight odor of methyl-*n*-amyl ketone is evident. Visible spore formation by *P. roqueforti* can be observed eight or ten days after manufacture. Fat hydrolysis by the natural milk lipase is evident during the manufacturing process but becomes more extensive after growth of *P. roqueforti* in the cheese. When mold becomes abundant in the cheese, the texture of the cheese becomes softer due to the production of a protease by *P. roqueforti.* The protease produced by mold, however, is not solely responsible for the decomposition of casein, since rennet as well as *S. lactis* can bring about appreciable breakdown. However, of the various proteases, that produced by *P. roqueforti* is perhaps the most important. Therefore, *P. roqueforti* contributes to protein degradation as well as to flavor production in blue-veined cheeses.

Flavor. Considerable research has been conducted to determine the compounds responsible for the flavor of blue-veined cheese. This flavor has been described by various individuals as sharp, peppery, pungent, burning, piquant, etc.

Orla-Jensen (15) stated that the chief constituent responsible for the flavor and aroma of Roquefort cheese is ethyl

butyrate. Currie (4) was of the opinion that the accumulation in the cheese of caproic, caprylic and capric acids with their easily hydrolyzable salts are responsible, in large part, for the peppery taste and burning effect of Roquefort cheese. Also, Currie (5) noted that Blue cheese made from sheep's milk had

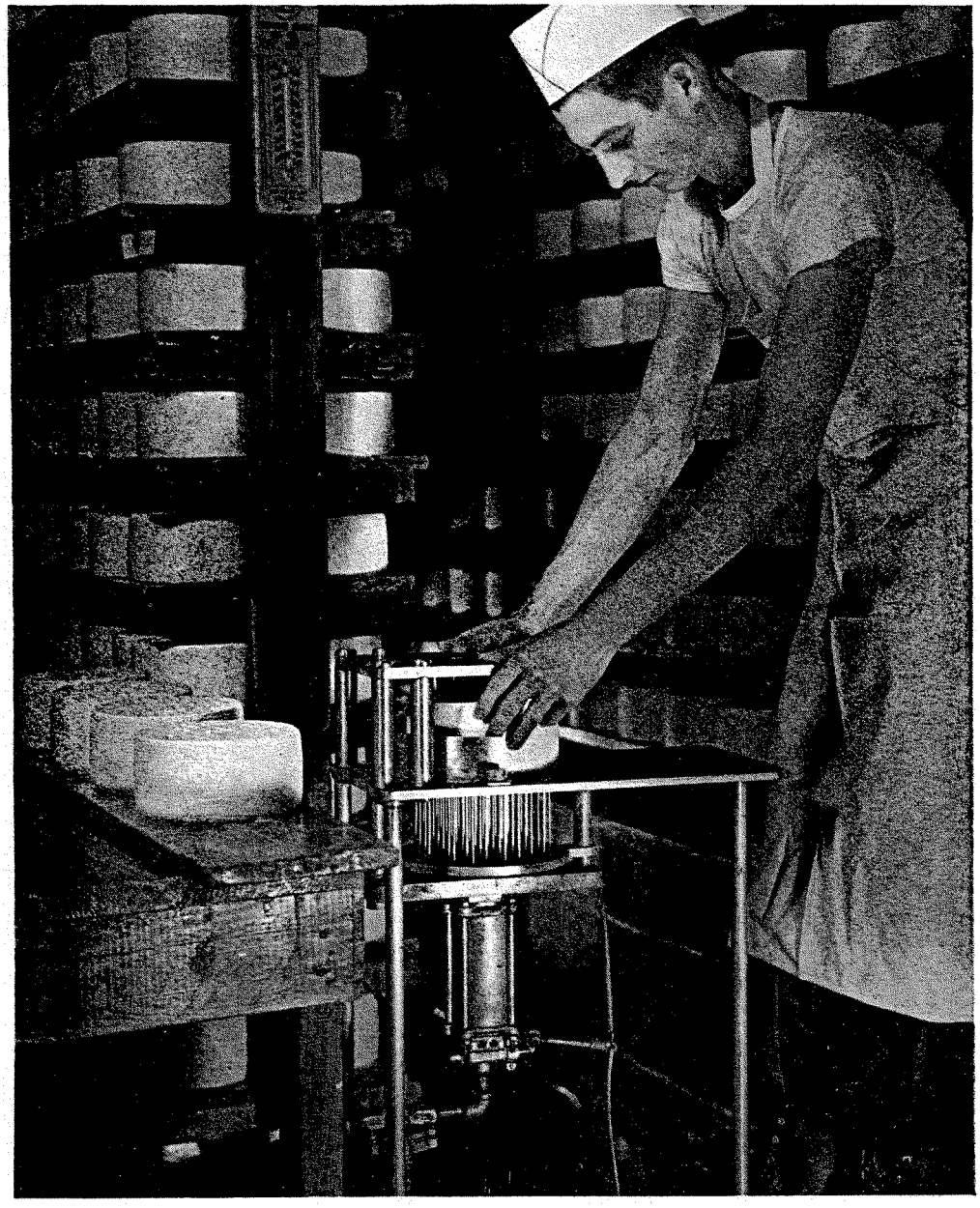


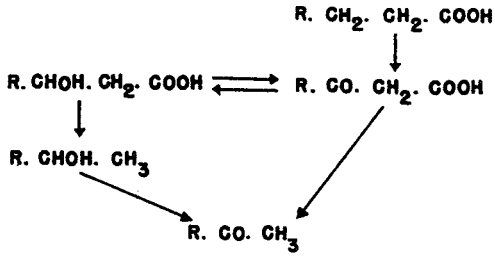
FIG. 3. Skewering blue cheese in order to promote rapid development of *P. roqueforti*. *(Photo by courtesy of Maytag Farms, Inc., Newton, Iowa.)*

more of the peppery taste than cheese of the same ripeness made from cow's milk.

Stärkle (17) studied the methyl ketones in the oxidative decomposition of certain triglycerides and fatty acids from the standpoint of rancidity of coconut fat. He considered the methyl ketones as possibly important in the rancidity of butter and in Roquefort cheese. Stärkle expressed the opinion that the characteristic aroma materials in the ripening of cheese by molds are, in the case of Roquefort, methyl ketones instead of esters. When he distilled Roquefort, about two drops of material was obtained which had an intensive odor of methyl-amyl and methyl-heptyl ketones. The amount was too small to permit separation and identification.

Bryant (1) demonstrated that *P. roqueforti* utilizes various fatty acids. When known amounts of fatty acids were added to a medium inoculated with *P. roqueforti,* there was reduction of the acids. The reduction was greatest with butyric acid and decreased as the molecular weight of the acids increased. Hammer and Bryant (9) stated that fatty acids alone do not account for the flavor of Blue cheese. These investigators added various fatty acids plus spores of *P. roqueforti* to sterile milk. A flask containing *n*-caprylic acid was of special interest. When held for several days at room temperature, no mold growth appeared at the surface, but the peppery odor of blue cheese was noted. Later, mold growth developed and the flavor disappeared. The odorous compound produced in milk with caprylic acid and mold spores added could be removed from milk by steam distillation. Also, the odorous compound could be removed from the distillate by shaking with ethyl ether. After evaporating the ether, the residue gave a ketone reaction. Various tests conducted on the residue led to the conclusion that the material was largely methyl-*n*-amyl ketone.

Stokoe (18) explains the formation of methyl ketones from fatty acids by molds as follows:



Recently, Patton (16) studied the methyl ketones of Blue cheese and their relation to its flavor. By means of a steam distillation and ether extraction procedure he was able to recover material from Blue cheese containing a high concentration of methyl ketones. Fractional distillation of this material gave relatively pure fractions of methyl-propyl, methyl-amyl and methyl-heptyl ketones. The methyl ketones appeared to be formed in the cheese by beta-oxidation of fatty acids to the beta-hydroxy acids and then to the beta-keto acids which are decarboxylated to form methyl ketones and carbon dioxide, according to the scheme outlined by Stokoe (18).

Influence of Certain Steps in the Manufacturing Procedure on the Activity of Fungi, Bacteria and Enzymes. Blue-veined cheese of the Roquefort type are produced largely from raw homogenized milk. When pasteurized milk is employed in manufacture, the natural milk lipase is destroyed and the resulting cheese require a longer period to attain a particular degree of flavor, even though homogenized-pasteurized milk may be employed. The effect of pasteurization is clearly brought out by the data in Table I.

Table I also shows the desirable effect of homogenization in bringing about in-

creased fat hydrolysis. From these data it is evident that increasing the fat surface by homogenization is more important in fat hydrolysis of Blue cheese than the presence of the natural milk lipase. However, the use of raw milk plus homogenization produces a very definite increase in fat hydrolysis when compared to either raw non-homogenized or pasteurized-homogenized milk. It is quite difficult to assess the relative importance of milk lipase and lipase produced by *P. roqueforti* with regard to their ability to hydrolyze fat. Mold development in cheese varies to some extent and greatly influences fat hydrolysis.

The practice of separating milk and of homogenizing the cream obtained from it rather than homogenizing the milk itself is followed because of a saving in time, a smaller sized homogenizer can be used, and the procedure is more adaptable to the bleaching operation. If 100 pounds of milk containing 3.5 percent fat is separated into skimmilk and cream containing 35 percent fat, only ten pounds of cream must be homogenized. When fat is bleached with benzoyl peroxide in order to produce white cheese, the temperature at which bleaching is carried out is often greater than the temperature required to destroy the natural milk lipase. The milk lipase is largely in the skimmilk portion when milk is separated. Therefore, the cream may be heated to temperatures which destroy milk lipase without affecting its activity in cheese appreciably.

The culture of lactic acid-producing streptococci added to the milk intended for cheese manufacture contains about 0.75 percent lactic acid. The small amount of lactic acid added by the culture plus a small amount of lactic acid produced by the culture during the ripening period greatly assists in the coagulation of milk by rennet. Ripening is carried out at 90° F because it is near the optimum growth temperature of the lactic acid-producing bacteria. The entire cheese-making operation is conducted at 90° F because growth and acid production by the lactic culture must be maintained. Production of acid also assists in the removal of moisture from the curd particles and lowers the pH to a point that is unfavorable for the growth of many microorganisms. However, development of too much acid during manufacture is not conducive to good quality cheese.

One of the most important factors in the production of good quality cheese is the ability of the cheesemaker to obtain a firm curd without an excessively high whey acidity. Acid favors fusion of the curd particles, and if the cheese have few mechanical openings in the interior, the mold will not be able to spread through it. Cheese with few mechanical openings shows little mold development and consequently will not ripen in a

TABLE I

EFFECT OF PASTEURIZING AND THEN HOMOGENIZING THE MILK INTENDED FOR CHEESEMAKING ON THE AMOUNT AND GENERAL TYPES OF VOLATILE ACID, THE ACID VALUE OF THE FAT, AND THE FLAVOR OF BLUE CHEESE (13).

Type of milk used	Age of cheese	Ml. N/10 volatile acids per 200 g. cheese	Acid No. of fat	Flavor
Raw	12 weeks	9.0	13.2	Poor, musty, bitter
Raw-homogenized	12 "	32.0	63.0	Good
Pasteurized-homogenized	12 "	15.0	27.6	Lacking in flavor

short period of time. Both flavor and texture will be influenced by the lack of mold growth.

The salt and oxygen content of Roquefort-type cheese are very important in controlling the growth of fungi other than *P. roqueforti*. The presence of four percent salt and a low oxygen tension inhibit the growth of all fungi except *P. roqueforti* or very similar species. *Oospora lactis*, the common mold associated with milk and cream, is one of the few molds capable of growing in a low oxygen tension, but it is inhibited by the salt concentration. If a cheese contains four percent salt and 40 percent moisture, the concentration of salt in the aqueous portion is ten percent. Some openings must be made in the cheese to permit satisfactory growth of *P. roqueforti*. This is accomplished by piercing the cheese with needles, and the process is known as skewering. Golding (6) presents data to show that the purpose of skewering blue-veined cheese is to allow carbon dioxide to escape rather than to permit entrance of air. If cheese are punched with needles having too great a diameter, the oxygen content in the punched areas will be great enough to permit the development of fungi other than *P. roqueforti*.

Defects Due to Fungi. Some manufacturers of Blue cheese have experienced considerable difficulty with red areas of mold growth on the surface during the ripening period or during the storage period. Red mold is very conspicuous on Blue cheese and first becomes evident as small bright red colonies which may be few or very numerous. In some cases the red mold is noted in about ten days. It appears on the surface and is important only because of its color. Hammer and Gilman (10) identified it as *Sporendonema casei* and suggested use of petrolatum containing added calcium propionate or propionic acid for coating the cheese and thus preventing development of the mold. At the present time the method employed for limiting growth of the mold consists of coating the cheese with a flexible cheese coating. The cheese can be punched (skewered) after a suitable coating has been applied.

When Blue cheese are punched with needles that are too large in diameter, molds other than *P. roqueforti* may invade through the punch holes. In some cheese-manufacturing plants, a black discoloration of cheese accompanied by a musty flavor has been noted. This defect originates at the area of the punch hole and often spreads through the interior of the cheese. Bryant and Hammer (2) studied the black-discoloration defect and found that it was caused by *Hormodendrum olivaceum*. Their studies indicated that the mold required a good oxygen supply for growth and therefore would not produce defective cheese if the cheese were manufactured properly.

A gray discoloration is sometimes noted in Blue cheese, particularly in cheese that are ripened for very long periods. The discoloration is first noted on the surface and gradually spreads toward the interior, accompanied by a mousy ammoniacal flavor which later becomes soapy. Bryant and Hammer (2) thought that the gray discoloration might be due to the action of certain forms of *Actinomyces* because of their tendency to darken media containing tyrosine. Also, certain cheese discolorations have been attributed to melanins produced by the action of tyrosinase upon tyrosine. The gray discoloration could not be produced by addition of *Actinomyces* cultures to cheese curd made into Blue cheese. Presumably the organisms failed to grow in the cheese.

Camembert Cheese

Camembert cheese had its origin in France about the time of Napoleon.

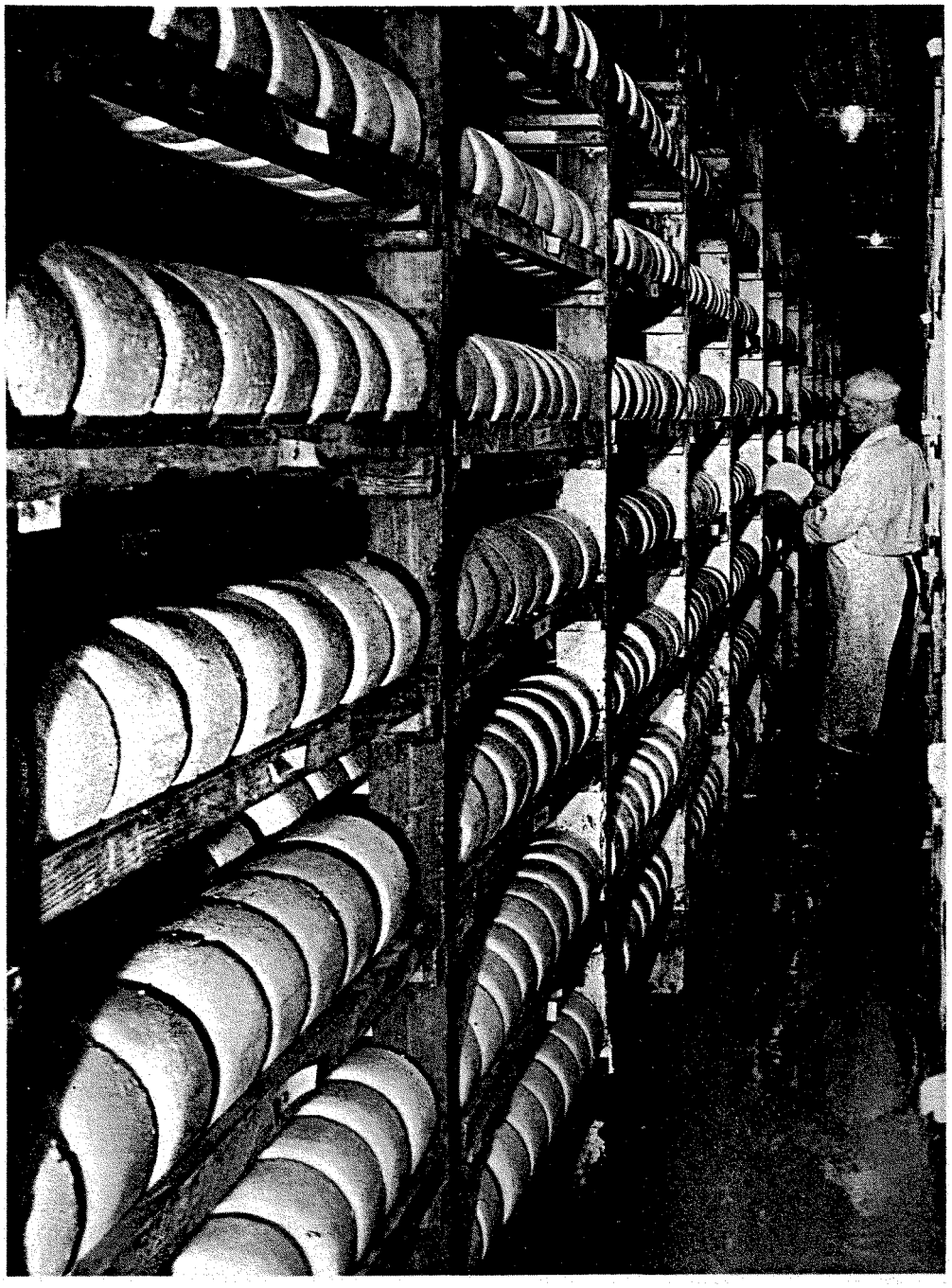
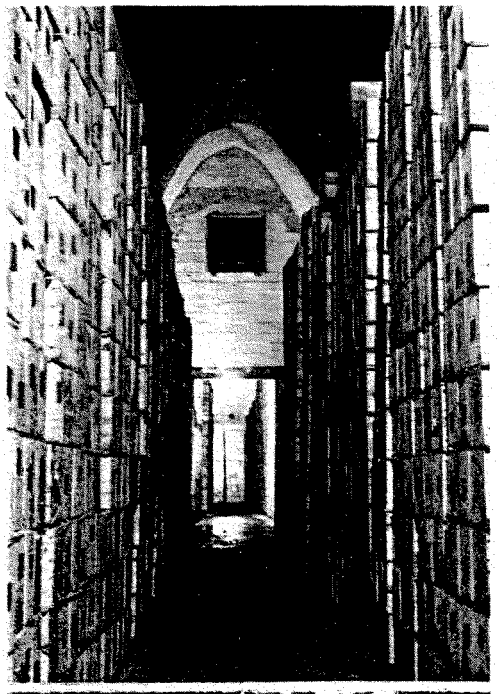


FIG. 4. A blue cheese curing room fitted with temperature and humidity controls. (*Photo by courtesy of Maytag Farms, Inc., Newton, Iowa.*)



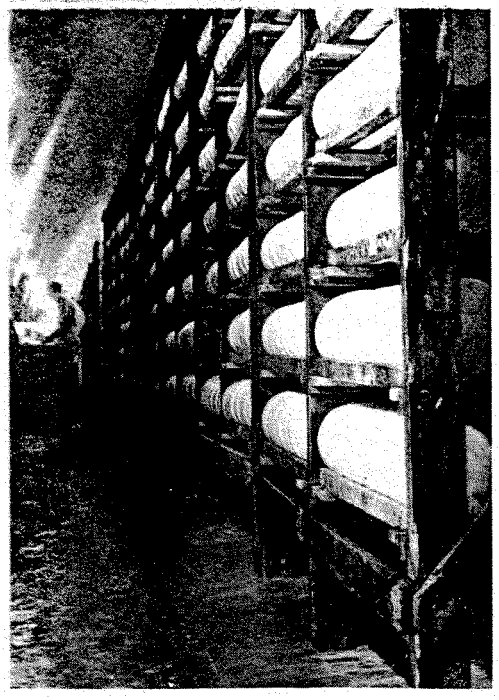


FIG. 5 (*Upper*). Man-made cave for the storage of blue cheese. (*Photo by courtesy of Treasure Cave Cheese Company, Faribault, Minn.*)

Some of the cheese was exported to the United States where it found a select market. About 1900, various cheese manufacturers and research organizations in this country became interested in Camembert. Much of the pioneer work with regard to the development of manufacturing procedures and studies on the chemistry and microbiology of it was conducted jointly by the Storrs Agricultural Experiment Station, Storrs, Conn., and the United States Department of Agriculture. The first research work on Camembert cheese in the United States was started about 1904. Shortly after this time, several commercial companies began manufacturing the cheese. Previous to 1920 a considerable portion of the amount consumed in the United States was imported from France, but at the present time, practically all of this type cheese in the United States is of domestic manufacture.

Manufacturing Procedure. Pasteurized milk containing 3.5 to 3.7 percent butterfat is adjusted to a temperature of 86° F, and two percent of an active culture of lactic acid-producing streptococci is added. The milk is held at 86° F about one hour to permit formation of lactic acid by the bacterial culture. After the ripening period the milk is curdled by addition of rennet extract which is added at the rate of three and one-half to four ounces per 1000 pounds of milk. The milk generally coagulates in 12 to 14 minutes, but it is allowed to remain undisturbed for about one hour to permit formation of acid and a desirable curd texture. After the setting period the curd is cut into cubes of about one-quarter inch by means of curd knives. The cut curd is not disturbed for about 15 minutes after cutting, and then it is gently stirred for about one hour to permit the curd to firm and the

FIG. 6 (*Lower*). A natural blue cheese curing room made by tunneling into a hill of St. Peter's sandstone. (*Photo by courtesy of Treasure Cave Cheese Company, Faribault, Minn.*)

acidity to increase. The vat contents are held at 84° to 86° F throughout the entire manufacturing operation. When the curd is sufficiently firm, a portion of the whey is drained and the curd is dipped into forms. The forms consist of perforated cylinders five inches in diameter and five inches high. Before dipping the curd, the forms are placed on reed mats about 15 inches square. The mats provide a surface which permits whey to escape and thus facilitates draining period the cheese are turned frequently to assure even distribution of mold. After ripening for ten to 14 days, the cheese are cut into wedge-shaped portions and wrapped in foil.

Preparation of Mold. The usual method of culturing mold for use in inoculating Camembert cheese is to grow the mold on water crackers. Water crackers are placed in a wide-mouth container and sterilized by means of steam under pressure. After steriliza-

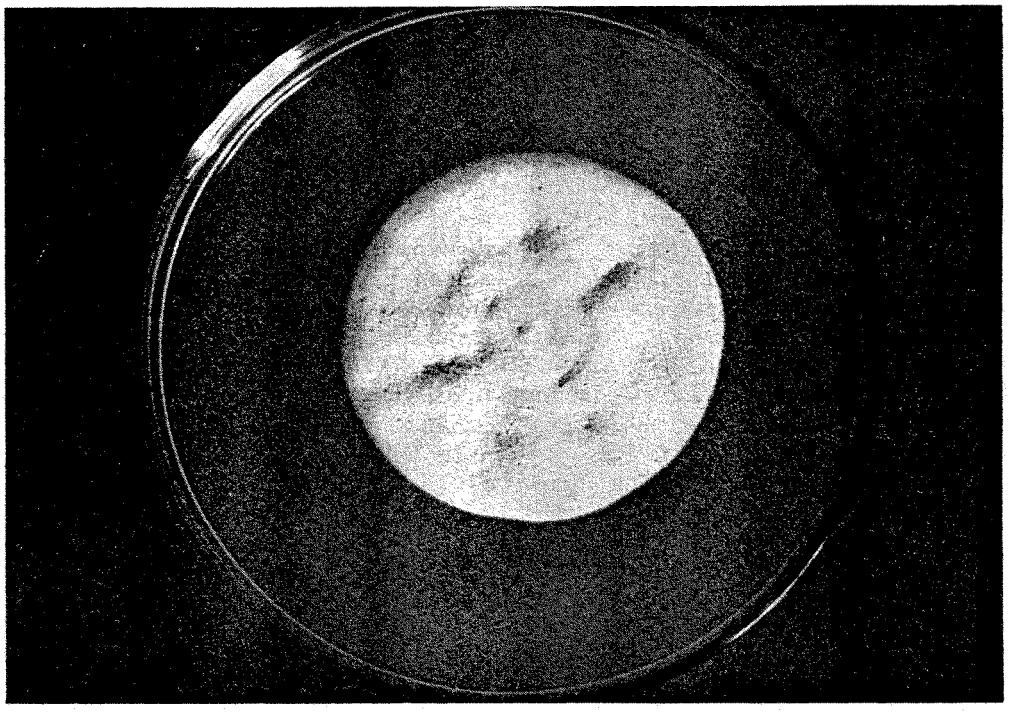


FIG. 7. *Penicillium camemberti* grown on Czapeks agar.

age. The cheese are turned in the hoops regularly after dipping to provide uniform drainage and regular shape. Eighteen to 20 hours after dipping, the cheese are removed from the hoops and taken to the salting room. The cheese are surface salted so as to contain approximately two and one-half percent salt. Following salting, the cheese are placed in the ripening room which is maintained at 56° to 58° F and a relative humidity of about 90 percent. During the ripention the crackers are moistened with sterile water and inoculated with a culture of *Penicillium camemberti* taken from an agar slant. The containers, filled about two-thirds with crackers, are incubated at 60° F until the crackers become covered with mold. The cheese are inoculated by sprinkling or spraying with a suspension of the mold spores. Inoculation of the cheese with mold is generally done immediately after taking the cheese from the hoops, although

some manufacturers prefer to inoculate the cheese during or after the salting. Both methods have given satisfactory results.

Role of Bacteria and Fungi. During the manufacture of Camembert cheese, the lactic acid-producing streptococci are important from the standpoint of acid production only and perform the same general function in Camembert as they do in blue-veined cheese. Acidity produced by these bacteria is necessary for curdling, whey drainage and properly textured curd. The lactic acid bacteria find conditions for growth very satisfactory during the manufacturing operation, and conditions are adjusted so that the organisms will grow rapidly. Large numbers of these bacteria are present in the interior of the cheese throughout the ripening period.

Some factories manufacturing Camembert cheese believe that the organism *Bacterium linens* is essential to the manufacture of good quality product, and the organism is added to the cheese milk or to the cheese surface after manufacture. *B. linens* requires considerable oxygen for growth and therefore appears only on the cheese surface. The organism is very salt-tolerant also and thus finds the cheese surface an ideal medium for growth. The exact role played by *B. linens* in the ripening process is not fully understood. It is known that this bacterium can neutralize the acidity at the surface and may assist the action of proteolytic enzymes which act more rapidly at a neutral or slight alkaline reaction. Most cheese will contain this organism on the surface, even though a culture is not added. In some cases the organism is not noted until late in the ripening period pecause of the early and rapid growth of *P. camemberti*. *B. linens* produces a reddish cast, and if the humidity of the ripening room is excessively high it will form a definite reddish slime on the cheese.

The important ripening agent of Camembert cheese is *P. camemberti*. This mold requires considerable oxygen for growth and is present on the cheese surface. The mold mycelium does not penetrate the cheese. *P. camemberti* is quite salt-tolerant. The mycelium of the mold is visible on the cheese surface about four days after manufacture, and the surface shows a trace of gray color due to the production of spores after about ten days. *P. camemberti* produces an active proteolytic enzyme which is largely responsible for cheese ripening. Although mold growth is confined to the surface, the proteolytic enzymes produced by the mold find their way to the interior of the cheese. Because of surface ripening, either Camembert or Brie cheese are manufactured so as to have a thickness of one to one and one-fourth inches. Thickness of the cheese is important because it is essential that the entire cheese be ripened uniformly. If a thicker cheese were inoculated with *P. camemberti*, its surface would be digested to a watery consistency, while the center would remain hard.

In the early studies on Camembert (19) it was believed that *Oospora lactis* was responsible for the flavor of the cheese. If *O. lactis* is present on the cheese surface it generally grows slowly during the early part of the ripening, due to the high salt concentration. However, it finds conditions suitable for growth late in the ripening period or at about the time that the cheese acquires typical flavor.

Defects. Since Camembert cheese is manufactured from pasteurized milk, many microorganisms capable of producing defects are destroyed by the pasteurization process if it is carried out properly. Certain defects may result from improper manufacturing procedures or from failure of the lactic acid-producing bacteria to form acid. However, the principal defects encountered

are due largely to contaminants on the surface. Growth of the white mold *P. camemberti* on the surface is very evident and is expected by the consumer. Contamination by blue-green molds, black molds, etc., presents an unsightly surface, and such contaminants are eliminated by proper cleaning and sanitizing methods in the cheese plant and in the curing rooms. Manufacturers of Camembert cheese prefer to use several small curing rooms rather than a large one so that the rooms can be emptied and cleaned before refilling with fresh cheese. Yeast contamination on the surface may result in the production of cheese with a yeasty flavor. Pink yeasts are particularly objectionable because they are readily visible on the surface.

With many cheese varieties, a long ripening period is quite desirable from the standpoint of flavor development and protein degradation. Camembert or Brie cheese cannot be ripened for extended periods and must be marketed in a rather short time. Extended ripening results in a cheese with too much protein decomposition and this is brought about by the protease of *P. camemberti.*

Summary

Fungi are necessary for the manufacture of two general types of cheese—blue-veined cheeses (Roquefort, Gorgonzola, Stilton and Blue); and Camembert and Brie cheese.

The important mold in blue-veined cheeses is *Penicillium roqueforti* or a very similar species. This mold tolerates considerable sodium chloride and will grow in a low oxygen tension. *P. roqueforti* produces a lipase which is important in contributing to the characteristic flavor of blue-veined cheeses and also produces a protease which aids in protein decomposition or in the production of cheese with a buttery texture.

The important mold on Camembert or Brie cheese is *Penicillium camemberti.* This mold grows well on the surface of cheese containing 2.5 percent sodium chloride. *P. camemberti* produces a very active protease which penetrates cheese and which is responsible for the extensive protein degradation of Camembert or Brie.

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Utilization Abstract

Violet Perfumes. The Parma violet, a cultivated form of *Viola odorata semperflorens*, is the most important source of violet perfume manufactured in France, where more than 30 species are native. "It is most at home in the regions of Grasse and Toulouse in France and in the Taggia Valley in Italy. The violets which are used in perfumery come mostly from Grasse; those from the other districts are principally used to make up bouquets. In 1865 Vence and its surroundings produced 80,000 kilograms of Parma flowers for perfumery purposes. Around 1870 the cultivation had spread to Le Bar, Tourettes, and Peimeynado".

"The violets which grow through the loose ground surrounding trees are more fragrant than those which are exposed to the open sun. For this reason, the plantations in the surroundings of Grasse are protected by olive trees and orange trees. Violets must also be protected from fresh winds and the rigors of wintertime, as these may retard the flowering or even kill the plant. In the plains of Hyères brier branches and in Italy straw mats are used as cover, and around Toulouse glass-covered frames form the protection".

"The time of planting varies according to the district and method of cultivation. In the dry climate of the Provence it must not be undertaken later than March–April; the best months are December and January. During planting, as well as during summertime, the ground has to be sufficiently watered. Besides this, it has to be ploughed and weeded. During picking time, the flowering has to be sustained by applying liquid manure".

"The violets begin to flower right during the first year after planting. The picking of flowers for bouquets starts during October–November, while the flowers to be used for perfume are gathered from January to April. Picking is done either in the morning, after the dew has fallen, or at night. This kind of harvesting takes much time and is costly, as the flowers have to be 'cut', one after the other, with the nail. A woman is able to collect three to six kilograms per day, according to ability and abundance of flowers. One kilogram has about 4000 flowers".

"The flowers have to be processed as soon as they are picked, if one wants to have a perfume which has not lost anything of its delicacy and its freshness. The violets are processed either by heat-method maceration in fat, or cold-method maceration in oil. However, the most common way of processing is by means of volatile dissolvants which make it possible to obtain a strong concentration within a very small space. Results vary according to the method used. About 1100 to 1200 kilograms of Parma violets must be treated in order to obtain one kilogram of the final product".

The Victoria variety, which is used in bouquets and at the end of the season in the factories around Grasse, is planted in the districts of Hyères, Solliès, Ollioules and Var, and on the Italian Riviera. The most popular variety for bouquets, however, is La Luxonne; two others for the purpose are Princess of Wales and The Czar. From 1400 to 1500 kilograms of Victoria violets are needed for one kilogram of perfume. (A. J. Hughes, *Am. Perf. & Ess. Oil Rev. 60: 105. 1952*).