

THE EFFECT OF PROCESS PARAMETERS
ON THE FLAVOR AND METHYL KETONE
CONTENT OF QUICK RIPENED
BLUE CHEESE

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ABSTRACT

THE EFFECT OF PROCESS PARAMETERS ON THE FLAVOR AND METHYL KETONE CONTENT OF QUICK RIPENED BLUE CHEESE

By

Sharon E. Albert

Quick ripened Blue cheese was modified during processing is an effort to improve the original method of Hedrick, Kondrup and Williamson (1968). It was felt that more control over the fermentation was needed in order to assure a better, more easily reproducible product. In an attempt to improve the cheese, the following processing variables were employed:

- 1) lowering the curing temperature
- 2) cooking the curd to 100° F
- 3) direct acidification with lactic acid
- 4) storage of the finished product at 40° F
for 2 weeks
- 5) use of a white mutant strain of Penicillium
roqueforti.

All samples were analyzed for methyl ketone content and total fat. The C₅ - C₁₃ methyl ketones were isolated and separated using column chromatography. The cheese was also evaluated organoleptically.

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The curing temperature was lowered to 50° F in an effort to reduce contamination by competing micro-organisms. Cheese ripened at this temperature was cured for 11 days and salted on days 7, 8 and 9 or 1, 9 and 10. In addition, the curd was cooked during manufacture to prevent shattering and facilitate drainage. The two cheeses made by this method were high in methyl ketone content and had excellent flavor. This procedure was suggested as a satisfactory alternative method of manufacture.

Quick ripened Blue cheese was made by direct acidification with lactic acid in an attempt to automate the process and reduce labor costs. Cheese made by this method did not develop typical Blue cheese flavor and color. Using the processing conditions described, this cheese was considered unsatisfactory.

Quick ripened cheese was also stored at 40° F for two weeks before analysis. The stored cheese had a much finer flavor and higher ketone content than the original. Such brief storage may be one way of standardizing flavor quality of various lots of cheese.

A white mutant strain of P. roqueforti was used in an attempt to produce a Blue cheese flavored product without the blue mold color. Cheese made with this mutant strain had a typical, although mild flavor. It was felt that the white cheese would be preferable for applications where the blue-green color of the mold is objectionable or offensive.

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INTRODUCTION

In recent years, cheese and cheese products have increased tremendously in popularity. The availability of salad dressings, dips and other products utilizing Blue and other cheeses has contributed to the increase in per capita consumption of natural cheese. As one means of improving production efficiency and to lower the cost of production, Hedrick, Kondrup and Williamson (1968) devised a method of quick ripening Blue cheese. This cheese is ripened in a loose curd form at 62° F in an atmosphere of at least 95% relative humidity. Less than two weeks are required for full ripening, and significant reductions in labor costs are realized in the process.

This project was undertaken to investigate the possibility of modifying and improving the quick ripening process since the flavor of quick ripened cheese was observed by Blakely to vary and to be atypical of a completely desirable Blue cheese. Several modifications of the quick ripening procedure were developed and this thesis is concerned with the analysis and evaluation of cheeses made by these procedures.

LITERATURE REVIEW

Cheese flavor is a complex phenomenon resulting from several factors. These include the treatment of the cheese milk, the organisms used, and the ripening conditions. Much work has been done in the past to gain a better understanding of the flavor of Blue mold cheeses. The complexity of the system is evidenced by the work of Day and Anderson (1965), where approximately 100 flavor compounds were identified in the volatile fraction of Blue cheese by gas chromatography and mass spectrometry. The major components were methyl ketones, secondary alcohols and esters of aliphatic acids.

The ripening products formed in cheese have been classified into two types by Harper and Kristoffersen (1956). Primary products (free amino acids, free fatty acids and lactic acids) are formed by lipolysis, proteolysis and glycolysis. Secondary and tertiary degradation products are formed by the enzymatic breakdown of the primary products. These compounds are β -keto acids, aldehydes and ketones, acetylmethylcarbinol and diacetyl, formed by transamination, deamination, decarboxylation and beta oxidation reactions.

Flavor Components of Blue Cheese and Their Origin

Free Fatty Acids

The degradation of milk fat forms a major source of flavor compounds in Blue cheese. In 1914 Currie reported that Penicillium roqueforti produced a lipase which causes hydrolysis of milk triglycerides. This worker found an increase in volatile acids during ripening of Roquefort cheese. He attributed the "peppery" or burning flavor of the cheese to the presence of caproic, caprylic and capric acids. Caproic, valeric and butyric acids were found in blue mold cheeses by Thomasow (1947). The "pungent, peppery flavor" of Blue cheese was attributed to free fatty acids and their oxidation products by Morris, Jezeski, Combs and Kuramoto (1963). Harper (1959) claimed that those fatty acids of medium to long chain length are predominant and that free amino acids also contribute to the flavor of Blue cheese.

Free fatty acids are important in the flavor balance of cheese. Parmalee and Nelson (1949) observed that when fatty acids were present in excessive amounts the cheese displayed "an undesirable rawness of flavor", possibly due to a change in the development of flavor. When butyric, caproic, caprylic, capric and lauric acids were added to Blue cheese, the resulting cheese showed a better flavor than a control cheese. However, a "smoothness and fullness" of flavor was lacking. Anderson (1966) has concluded

that the overall flavor of Blue cheese does not depend on the total volatiles present, but on the relative amounts of the individual constituents.

Of the fatty acids present in Blue cheese, many may still be unknown. Magidman, Herb, Barford and Reimenschneider (1962) conducted a study of the free fatty acids in cows milk. At least six fatty acids were identified, some for the first time. Utilizing gas chromatography, Day and Anderson (1965) identified the major free fatty acids (C_2 - $C_{18:3}$) in domestic Blue cheese. No formic, propionic or isovaleric acid was evident in any of the cheese samples analyzed. These workers postulate that from 75 to 85% of the acid present in cheese may exist in the salt form. The salts of long chain acids are soaps and may give a characteristic flavor to Blue cheese.

Blakely (1970) reported finding the same mole percent free fatty acids in quick-ripened blue cheese as reported in a commercial sample analyzed by Day and Anderson (1965).

Lipase Activity

The enzymes involved in lipid hydrolysis in cheese originate from two sources:

1. Milk lipases
2. Microbial lipases (Stadhouders and Mulder, 1957)

The presence of a multiple lipase system in milk has been demonstrated several times. Albrecht and Jaynes (1955) indicated that two lipase systems exist in raw skim

milk with optima at pH 5.4 and 6.3. Two different lipase systems were also observed by Tarassuk and Frankel (1957). One lipase was irreversibly absorbed on the fat globule membrane when milk was cooled, the other was associated with casein. Harper and Gaffney (1970) concluded that lipase is an absorbed entity on various caseins. These workers also presented evidence for a multicomponent lipase system in milk.

When mixed triglycerides are hydrolyzed, there is an obvious preference for the fats containing short chain acids (Jensen, 1964). Milk lipase preferentially releases fatty acids from the primary positions of a triglyceride. Since butyrate appears to be predominantly a primary ester, an apparent preferential release of butyrate results. Harwalker and Calbert (1961) reported that lauric and higher acids were the major acids released at all stages of lipolysis, but as lipolysis progressed the ratio of these acids to butyric decreased. Very little change was observed in the mole percentages of the other acid fractions.

The microbial lipase present in Blue cheese originates mainly from Penicillium roqueforti. Spores of this mold are added to the cheese milk or to the curd. Morris and Jezeski (1953) studied the lipolytic activity of a mycelial preparation of P. roqueforti. Optimum activity was obtained between pH 6.0 and 6.7 on tributyrin and pH 7.0 to 7.2 on butterfat. Reports by Inamura (1963a) and

Inamura and Kataoka (1963b, 1966) indicate two types of lipase with optima at pH 6.5 and 7.5. Maximum lipolytic activity on a 5% butteroil substrate was obtained at pH 8.0 and 37° C by Eitenmiller, Vakil and Shahani (1970). Lipase activity was stimulated by $MnCl_2$ and $MgCl_2$. The enzyme hydrolyzed tributyrin, tricaprylin, tripropionin and triolein in decreasing order. Wilcox, Nelson and Wood (1955) found butyric, caproic and caprylic acids selectively released in reaction mixtures of butterfat and P. roqueforti lipase.

Some lipolytic activity in Blue cheese may be due to starter culture activity. Willart and Sjostrom (1959) claimed starter organisms did not contribute to lipolysis. However, Fryer and Reiter (1967) found a weak lipolytic activity in seven out of fifty-six tested strains of Streptococcus lactis.

Volatile Acidity

There may be a relationship between flavor and volatile acidity in Blue cheese. Parmalee and Nelson (1949) found that cheese scoring high in flavor generally had a volatile acidity of 30 to 50 ml of 0.1 N acid per 100 g of cheese. However, no correlation was found between fat acidities and flavor scores. Lane and Hammer (1938) found a relationship between volatile acidities, fat acidities and flavor scores. Cheese with high volatile acidity and high fat acidity had more characteristic

flavor than low acid cheeses. Also, cheeses made from homogenized milk had two to four times the volatile acidity of cheeses made from non-homogenized milk.

The acidity of Blue cheese was studied by Coulter, Combs and George (1938b). A minimum pH of 4.7 was attained within 24 hours after manufacture. The pH increased gradually to 6.5 (in approximately three months) and then decreased to 5.7 by the ninth month of ripening. The marked rise in pH from about 2 weeks to 10 weeks was due to continuing proteolysis (Currie, 1914; Morris, Combs and Coulter, 1951). Morris (1963) noted a tendency for the rate of rise of pH to decrease as the pH approached 6.5. The lipase systems in Blue cheese were active in the pH range occurring throughout curing.

Methyl Ketones

Starkle (1924) obtained heptanone-2 and nonanone-2 by distillation of Roquefort cheese. He postulated that these methyl ketones were formed by the metabolism of fats by Penicillium roqueforti and that they were responsible for the flavor and aroma of the cheese. Later workers (Patton, 1950; Morgan and Anderson, 1956) identified ketones in Blue cheese using steam distillation and chromatography. Pentanone-2, heptanone-2 and nonanone-2 were isolated from samples with aromas considered to be typical of Blue cheese.

The work of Coffman, Smith and Andrews (1960) showed the major neutral components of Blue cheese to be heptanone-2, nonanone-2, undecanone-2, heptanol-2, nonanol-2 and an unidentified ketonic constituent. Coffman also reported the major acids as butanoic, pentanoic, hexanoic, octanoic and decanoic. These flavor constituents were isolated by vacuum distillation and identified by infrared spectroscopy and chromatography.

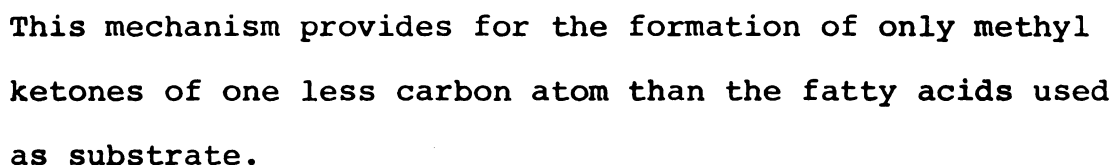
Nawar and Fagerson (1962) reported finding the following flavor constituents in Roquefort cheese: pentanone-2, heptanone-2, nonanone-2, acetaldehyde, isobutyraldehyde, butanone-2, propanol-2, diacetyl, hexanone-2, pentanol-2, octanone-2 and heptanol-2. Niki, Yoshioka, and Ahiko (1966) also identified acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and undecanone-2 in Blue mold cheeses.

It is generally agreed that heptanone-2 is the major ketone present in Blue cheese (Anderson, 1966; Schwartz and Parks, 1963). Schwartz and Parks found only small amounts of methyl ketones with chain lengths of more than nine carbon atoms. The formations of methyl ketones did not appear to be in a definite ratio or related to the age of a cheese. Blakely (1970) found nonanone-2 to be the major methyl ketone in samples of quick-ripened blue cheese.

Stokoe (1928) believed that the formation of methyl ketones was the second step in an "abnormal" oxidation of fatty acids. From work with Penicillium palitans and

Penicillium glaucum using coconut oil as a substrate, Stokoe concluded that fatty acids had a "narcotic" effect which caused a decarboxylation reaction. Starkle (1924) attempted to determine the origin of methyl ketones distilled from Roquefort cheese. Aspergillus niger, A. fumigatus and P. roqueforti when grown for several weeks in pure culture, with individual fatty acids as the carbon source, produced methyl ketones of one less carbon atom than the fatty acids. A mechanism advanced for the formation of these ketones was the oxidation of the fatty acid to the beta-keto acid followed by decarboxylation of the beta-keto acid.

Using C^{14} labeled octanoate, Gehrig and Knight (1963) confirmed the original assumption by early workers that β -keto acids are formed from free fatty acids and then decarboxylated to form methyl ketones. The 2-heptanone synthesized was unlabelled and $^{14}CO_2$ was produced. Lawrence (1966) found that 2-heptanone formed from 2- ^{14}C -octanoic acid was radioactive and that some radioactive carbon dioxide was also present. Therefore, ketone formation and beta-oxidation proceed simultaneously. The 2-C moiety (presumably acetate) formed by beta-oxidation is further oxidized in the tricarboxylic acid cycle. The pathway of fatty acid oxidation and methyl ketone formation may be outlined by the following scheme (Hawke, 1966).



- 1) lipolysis of milk triglycerides to form free fatty acids
- 2) formation of β -keto acids by oxidation of free fatty acids
- 3) decarboxylation of β -keto acids to produce methyl ketones
- 4) reduction of ketones to secondary alcohols.

In work with spores of P. roqueforti, Gehrig and Knight (1958) claimed that spores of mycelium, formed methyl ketones. Sporulation was necessary for the formation of ketones and the amount of sporulation was directly related to ketone formation. Germinated spores did not have the ability to form ketones. A direct relationship was also found between the amount of oxygen used by the spores and ketone production. No ketone was produced under anaerobic conditions. Girolami and Knight (1955) have shown that the oxygen uptake of spores is related to fatty acid chain length. In later work (1963) Gehrig and Knight stated that the spore is the active site for the metabolism of fatty acids to ketones of one less carbon. The ability of spores to form methyl ketones disappeared rapidly as germination progressed. There are conflicting reports on the ability of mycelium to produce ketones. Contrary to the conclusions of Gehrig and Knight, Lawrence and Hawke (1968) observed the oxidation of low concentrations of fatty acids over a wide range of pH values by the mycelium of P. roqueforti.

Lawrence (1966) identified methyl ketones produced by washed spores of P. roqueforti in a medium containing free fatty acids. The results showed that the higher the concentration of fatty acid, the longer the time required before the corresponding methyl ketone could be detected. The optimum acidity for ketone formation was between pH 5.5 and 7.0, depending on the concentration of the fatty

acid being oxidized. The maximum formation of heptanone-2 occurred at 27° C. Lawrence also observed that the ability of the spores to produce ketones decreased with age. A lag phase preceded the oxidation, which was lengthened with decreasing spore concentration and increased fatty acid concentration.

Girolami and Knight (1955) obtained results showing that the restriction or stimulation of the formation of ketones is dependent on the amount of fatty acid present. According to Anderson (1966) the amount of methyl ketone produced is not related directly to the amount of corresponding fatty acid available. The conversion of fatty acids seemed to be related to the chain length. Octanoic acid is converted to methyl ketone most rapidly, while butanoic and dodecanoic are the least rapid.

Dartey and Kinsella (1971) have studied the formation of methyl ketones in Blue cheese during ripening. The amount of the individual ketones fluctuated over the ripening period, with the exception of heptanone-2 and nonanone-2.

They were observed to be the two major methyl ketones, accounting for between 50 and 75% of the total. These workers believe these to be the two major flavor components of Blue cheese. During the initial days of ripening the methyl ketone content was extremely low, but increased to 120.4 μmol per 10 g of dry cheese on day 70. After day 70 there was a slow decline. No correlation was found between

the concentrations of individual methyl ketones. This may be due to interconversion of methyl ketones and secondary alcohols. Schwartz and Parks (1963) stated that the age of a cheese does not necessarily reflect its ketone content. Niki et al (1966) used a highly lipolytic variety of P. roqueforti for the production of Blue cheese. They noted that the ketone content increased rapidly after 7 weeks and reached a maximum at 21 weeks, when ketone flavor became evident.

Dartey and Kinsella (1973) studied the oxidation of palmitic acid by spores of P. roqueforti. Carbon dioxide was formed, along with carbonyl compounds, including a homologous series of C₃ to C₁₅ methyl ketones. The optimum yield of carbonyl compounds was obtained with a spore concentration of 6.3×10^8 spores/ml in the presence of D-glucose, at pH 6.5 and 30° C.

The inhibition of respiration and formation of methyl ketones by free fatty acids has been studied by several workers. Girolami and Knight (1955) stated that fatty acids inhibited a culture of P. roqueforti. The acids became increasingly toxic with greater chain length. Later, Gehrig and Knight (1963) found that the amount of inhibition depends on the pH of the medium, the concentration of free fatty acid and the chain length.

Dwivedi and Kinsella (1974) cultured mycelium of P. roqueforti in lipolyzed milk fat. Their results showed that low concentrations of fatty acids were metabolized

after a 6 hour lag period and higher concentrations required a longer (12 hour) lag, but greater total carbonyls were produced after 48 hours. They concluded that the higher concentration of fatty acids have only a temporary effect. The fatty acid concentration also influenced the relative proportion of methyl ketones produced. Pentanone-2 and undecanone-2 were dependent on the concentration of fatty acids, appearing only when the C_8 and C_{10} fatty acids decreased.

Other Carbonyls

Neutral carbonyls have been identified as flavor components of Blue cheese and subsequently studied by many researchers. Stokoe (1928) identified secondary alcohols among the products resulting from the action of P. roqueforti on coconut oil. He postulated that these compounds were the result of decarboxylation of β -hydroxy fatty acids, but considered the possibility that they were formed by the reduction of ketones. Pentanol-2, heptanol-2 and nonanol-2 were isolated by Jackson and Hussong (1958). They claimed that these neutral volatiles were probably formed by a reduction of the corresponding methyl ketones, because they did not appear until considerable amounts of ketones were produced. Day and Anderson (1965) indicated that under anaerobic conditions this reaction is reversible and could be responsible for the nonuniform distribution of individual ketones. These workers found

that spores of P. roqueforti incubated in phosphate buffer interconverted 2-pentanone and 2-pentanol. Mycelia suspended in media which prevented sporulation likewise caused this type of interconversion. Some yeasts associated with normal Blue cheese flora may also possess the ability to reduce methyl ketones to secondary alcohols. This was postulated by Anderson (1966) who also observed that the secondary alcohols appear to be present in Blue cheese in the same ratio as methyl ketones.

Acetaldehyde has been identified in Blue cheese (Bassett and Harper, 1958; Morgan and Anderson, 1956; Nawar and Ferguson, 1962). Propionaldehyde and isobutyraldehyde have also been detected by Nawar and Ferguson. Day and Anderson (1965) isolated furfural from Blue cheese.

Other compounds such as ethanol, acetylmethyl carbinol and diacetyl were documented in Blue cheese by Bavisotto, Rock and Lesniewski (1960), and Bassett and Harper (1958). Day and Anderson (1965) identified methanol, ethanol, n-pentanol, 2- and 3-methyl butanol and 2-phenylethanol in Blue cheese. These workers also identified the methyl esters of the even numbered carbon fatty acids C_2 through C_{12} . The ethyl esters of the even numbered carbon fatty acids acetic through decanoic were also present.

Proteolysis

The protein degradation of Blue cheese varies with the strain of P. roqueforti, the pH, and the temperature.

Niki et al (1966) detected an intra-cellular and an extra-cellular protease, both having an optimum pH of 5.5. The extra-cellular protease had a narrow pH range and contributed to casein break-down only during early stages of ripening. The intra-cellular protease, with a pH range of 5.5 to 7.0 contributed to proteolysis during the entire ripening period. The optimum pH for proteolysis was identified as 5.5 to 6.0 by Niskikawa (1958). An optimum temperature of 40° C for a 0.6% casein medium was found. Salt at a level of 2% slightly stimulated proteolysis, while 4% caused a decrease. Inamura (1960b) found maximum proteolysis at 42° C and pH 5.5. Starter organisms were found to have little proteolytic activity but did stimulate the growth of P. roqueforti.

High proteolytic strains of P. roqueforti showed low lipase activity and vice versa, according to the work of Niki et al (1966). Salvadori and Salvadori (1967) also illustrated this, as indicated by lipolysis of palmitic, oleic, stearic, azelaic and pelargonic acids. Sato, Honda, Yamada, Takada and Kawanami (1966) and Morris, Jezeski, Combs and Kuramoto (1955) studied the tyrosine content of Blue cheeses. The work of Sato et al showed the tyrosine content to be higher in well-ripened cheese, the content increasing slowly during ripening. Morris et al found no significant difference in tyrosine levels in cheeses made from raw milk, homogenized milk, and pasteurized milk. Modler, Brunner and Stine (1974) compared the

extra-cellular proteolytic activity of three strains of P. roqueforti and isolated the enzyme from the most proteolytic strain. The extracellular protease had a pH optimum of 3.0 and 5.5 for bovine serum albumin and casein, and a temperature optimum of 46° C.

Effect of Processing Variables on Flavor

Temperature

In Roquefort, France, the "cave" temperature is maintained at 43-46° F with a high humidity. For U.S. manufactured Blue cheese, temperatures of 48-55° F with 90-95% relative humidity have been reported.

Peters and Nelson (1961) studied the quality of cheese produced at different ripening temperatures. The highest quality cheese was produced by ripening for four weeks at 50° F and then four weeks at 76° F. Cheese ripened for eight weeks at either of these temperatures was of much lower quality. Morris (1969) used ripening temperatures of 37 to 60° F for Blue cheese. His results showed that higher temperatures are conducive to faster breakdown of fat and protein but the body, color and flavor of the cheese are of lower quality. Morris concluded that the best combination of ripening temperatures is high temperature initially and lower temperature in the last stages of ripening.

Salt

Most organisms cannot survive salt concentrations greater than about 6 percent, and essentially none survive a concentration of 10 percent (Morris, 1969). P. roqueforti can grow at salt concentrations up to about 16 percent brine. A 10 percent brine is the concentration maintained in Blue-veined cheeses. Therefore, a salt concentration of approximately four percent in a cheese of 40-44 percent moisture will provide the proper brine concentration to discourage contaminating molds, yeast and bacteria from growing.

The effect of salt content on flavor compounds in Blue cheese was studied by Willart and Sjostrom (1959) and Poznanski, Jaworski and D'Obyrn (1966). Willart and Sjostrom used 1.5-4.5% NaCl in making cheese. The greatest difference in the amount of fatty acids produced was detected at the lower salt concentrations. The higher the salt content used, the less higher chain length acids were observed. However, the ratio of butyric to higher acids remained the same regardless of salt concentration. Poznanski et al found that from salt contents of 3.0 to 3.9% the fat acidity increased with increasing salt content. Morris and Jezeski (1953) observed that the lipase activity of P. roqueforti decreased with increases in salt concentrations.

This was also observed by Inamura (1963). The lipolytic activity of P. roqueforti was decreased by greater

than 3% NaCl. The consumption of volatile fatty acids was decreased as salt concentrations greater than 4%.

Poznanski, Jaworski and D'Obyrn (1966) reported that acid production in Roquefort cheese diminished as the salt content increased from 3.0 to 3.9%. It was observed by Nelson (1970) that carbonyl production by spores of P. roqueforti is enhanced by the addition of salt. However, Dwivedi and Kinsella (1974) claimed that salt caused a prolonged lag phase before carbonyl production by mycelium. The relative proportion of the major methyl ketones was not significantly different.

Lane and Hammer (1938) found that that delayed salting to hasten mold development was generally unsatisfactory. A yellow color developed in the cheese which increased with the number of days between manufacture and salting.

Salt can also inhibit milk lipase in raw homogenized milk and cream (Gould, 1941). Levels of 5 to 8 percent salt were sufficient to inhibit lipolysis almost completely.

Acidity

High acidity is required for good mold growth in Blue-veined cheeses. Thom and Matheson (1914) and Matheson (1921) recommended that milk be brought up to an acidity of .23% before the addition of rennet. This was claimed to prevent off flavor development and soft curd.

According to Coulter et al (1938a) the pH of Blue cheese is influenced by the acidity of the cheese at setting and the acidity of the whey at dipping. If the acidity was too high during manufacture, the cheese developed an acid flavor, tough curd and crumbly body. Low acid cheese has soft curd and a tendency toward off flavors. In another paper (1938b) Coulter stated that experimental Blue cheese reached a maximum acidity of pH 4.7 within one day after manufacture. The lowest acidity, pH 6.5, was reached after about three months. Nesbitt (1953) obtained results showing the maximum acidity to be pH 5.6 after eight weeks of ripening.

Morris (1963) noted a tendency for the rate of rise of pH to decrease as the pH approached 6.5. The lipase systems in Blue cheese were active in the pH range occurring throughout curing.

The oxidation of free fatty acids to methyl ketones by P. roqueforti has also been found to be pH dependent. Lawrence and Hawke (1968) studied this conversion by mycelium and found the optimum pH to be dependent on the concentration of the acid, varying with each fatty acid. Mycelium grown at high pH (6.8) metabolized fatty acids faster than mycelium grown at pH 4.0. Similar conclusions were reached by Dwivedi and Kinsella (1974) but the effect of pH was only temporary. Also, as long as the pH was kept between 6 and 7 there was no significant difference in carbonyl production. Gehrig and Knight (1963) found that

the maximum conversion of octanoate to heptanone-2 at 25-30° C occurred at approximately pH 7. The maximum oxidation of sodium palmitate into carbonyl compounds was found to occur at pH 6.5, using a spore concentration of approximately 6.3×10^8 /ml at 30° C (Dartey and Kinsella, 1973).

Variations in the Manufacture of Blue Cheese

Much work has been done to modify the production of Blue cheese and Blue cheese flavorings. Knight, Mohr and Frazier (1950) used a mutant white strain of P. roqueforti to produce Blue cheese without a green-mold color. The mutant was produced by the irradiation of spores. This type of cheese was developed for use in the manufacture of Blue cheese flavored spreads and dips where lack of color is desirable. The white mold showed no significant changes in lipolytic and proteolytic activity, but the cheese had a milder flavor and softer, creamier body than traditional Blue cheese.

A Blue cheese flavored salad dressing was developed by Patton (1951). The volatile flavor components were extracted with salad oil from the fat of melted cheese. Other uses for the extract were suggested, including sauces, soups, dips and spreads. Patton also proposed the use of a synthetic cheese flavor consisting of 1 part methyl-n-amyl ketone to 20 parts butyric acid as a fortifying agent for natural cheese flavor.

Cort and Riggs (1967) have developed a method for producing Roquefort and Camembert type cheeses by adding enzymes extracted from P. roqueforti and P. camemberti during the manufacture of the cheese.

Nelson (1970) developed a 24-72 hour submerged fermentation to produce Blue cheese flavor of a high intensity. P. roqueforti was cultured in a sterile milk base medium. The ketone profile of the flavor, as identified by gas liquid chromatography, is similar to that of Blue cheese.

Research has been done on direct acidification of cheese curd, without the use of a starter culture. Shehata and Olson (1967) developed a direct acidification method using hydrochloric acid or lactic acid. Raw milk at 4.4° C was acidified to pH 5.9, 5.6, 5.0, 4.85, or 4.7, and then heated and coagulated with rennet. Curd adjusted to pH 5.6 produced the best curd, as judged by the ease of cutting and amount of shattering. Hydrochloric acid was more desirable, because the lactic acid curd was too soft, too high in moisture and difficult to handle during draining. Singh and Kristofferson (1972) produced a cheese flavor using a slurry prepared from directly acidified lactic acid curd. The slurry was inoculated with 5% lactic culture supplemented with glutathione, cobalt, riboflavin and diacetyl. The curd, which was used for the study of off flavor development, had physical characteristics comparable to cheese made by conventional methods, but

failed to develop characteristic cheese flavor during storage. Chanet (1972) patented a procedure for the acidification of soft cheeses by direct acidification with lactic acid. Whey from the coagulation stage was acidified and heated to 100° C to precipitate proteins and mineral salts, destroy acidifying microorganisms and inactive enzymes. The treated whey was in amounts sufficient to achieve the required pH.

Hedrick, Kondrup and Williamson (1968) developed a method of quick-ripening Blue cheese which involves ripening in a loose curd form at 62° F and greater than 95% relative humidity for ten days. This method is more economical than the production of conventional cheese. It is also faster and minimizes the formation of slime. Slime growth is reduced by the rapid mold growth. Moisture control is important and salting is controlled by the degree of ripening. According to Kondrup and Hedrick (1963) judges comparing quick-ripened Blue cheese to conventional Blue cheese determined that the body and flavor of quick-ripened cheese was equal or superior to conventional cheese. A modification of this method was used by Blakely (1970). Coconut oil was used to replace milk fat. High free fatty acid and methyl ketone contents were evident in the flavor of the cheese, but the flavor was considered to be atypical.

Lundstedt and Yau-Yee Lo (1973) have developed a method to prepare a Blue cheese type product from soy bean milk fortified with non-fat milk solids. This cheese also ripens in about two weeks.

EXPERIMENTAL METHODS

Quantitation of Methyl Ketones

Solvent Purification

Benzene: Carbonyl free benzene was prepared by refluxing reagent grade benzene with 1 gm of 2,4-dinitrophenylhydrazine (2,4-DNP) per 500 ml for 1-2 hours and redistilling.

Chloroform: Refluxed over KOH for 30 minutes and redistilled.

Nitromethane: Redistilled over boric acid and stored in a brown bottle.

Acetonitrile: Distilled and 81-82° C fraction collected.

Methanol: Refluxed over KOH for 30 minutes and redistilled.

Hexane (Carbonyl free): High purity hexane was redistilled and treated for the removal of carbonyls by the methods of Hornstein and Crowe (1962) and Schwartz and Parks (1961). A column was prepared using 40 ml of concentrated sulfuric acid blended with 75 gm of Celite 545 (Johns Manville). A small amount of anhydrous sodium sulfate was placed at each end of the column to serve as a dessicant. The eluate from this column was passed over a

column of Celite, analytical filter aid (Johns Manville) impregnated with DNP-hydrazine, phosphoric acid and water. The hydrazones were removed by slurrying with Sea Sorb 43 (Iodine Number 80; Fisher Scientific Company, Pittsburgh) and filtering as suggested by Blakely (1970).

Isolation of Methyl Ketones

The quantitative procedure for the isolation of methyl ketones was similar to that described for fats and oils (Schwartz, Haller and Keeney, 1963) and adapted to the analysis of Blue cheese by Anderson (1966).

Fat Extraction from Cheese Sample: Fifteen grams of Celite 545, dried for 24 hr at 150° C, and 10 g of cheese were thoroughly ground together with a mortar and pestle. The mixture was packed into a 25 x 500 mm chromatographic column, fitted with a fritted glass filter. The fat was extracted by passing 300 ml of carbonyl free hexane through the column and collecting the eluate. To determine the yield of fat, a sample of the same cheese was extracted as above and the eluate collected in a 300 ml Erlenmeyer flask. The hexane was evaporated on a steam bath under a stream of nitrogen. The fat was quantitatively transferred with ethyl ether to a pre-weighed Mojonnier fat dish. The solvent was evaporated and the dish re-weighed.

Formation of Dinitrophenylhydrazones (DNPH): The hexane-fat extract was passed through a reaction column of Celite, analytical grade, impregnated with 2,4 DNP,

phosphoric acid and water, to convert the monocarbonyls present in the fat extract into DNPH (Schwartz, et al, 1963). The column was first flushed with hexane until the effluent had the same spectral properties as pure hexane passed through a blank column. The sample was passed through, and the column was again flushed with hexane until the effluent had the same spectral properties as the hexane which had been passed through the column prior to sample addition. The hexane was then evaporated on a steam bath under a stream of nitrogen.

Removal of Fat from the DNP-hydrazones: The lipid material was separated from the DNPH by the procedure of Schwartz et al (1963) as modified by Anderson (1966). Fourteen grams of Sea Sorb and 28 g Celite 545 (dried 24 hrs at 150° C) were slurried in hexane and poured into a 2.8 cm ID chromatographic column equipped with a fritted glass filter and a 500 ml solvent reservoir. The column was packed using 3-5 psi nitrogen pressure and the fat-hydrazone mixture was dissolved in hexane and applied to the column. The fat was flushed from the column by the successive additions of 200 ml hexane, 100 ml hexane-benzene mixture (1:1, v/v) and 200 ml benzene. The hydrazones were eluted with 175 ml of a chloroform nitromethane mixture (3:1, v/v). The solvent was removed from the sample by evaporation on a steam bath under a stream of nitrogen.

Removal of Ketoglyceride DNPH: A column of weak alumina (Schwartz and Parts, 1961) was used to remove DNPH of ketoglycerides from the monocarbonyl derivatives. The sample from the preceding step was dissolved in hexane and applied to a 7.5 g column of partially deactivated alumina. The monocarbonyl fraction was eluted with 100 ml of a benzene-hexane mixture (1:1, v/v). The solvent was evaporated on a steam bath under a nitrogen stream.

Fractionation of Monocarbonyl Derivatives: The monocarbonyl residue from the alumina column was dissolved in hexane and applied to a 10 g Celite 545-Sea Sorb 43 (1:1, w/w) column. The Celite 545 was dried at 150° C for 24 hr. Separation of the methyl ketones from other DNPH classes was accomplished using a modification of the sequence of solvents suggested by Boyd, Keeney and Patton (1965). The following sequence of solvents was used: 50 ml quantities of 15, 25, 40, and 60 percent chloroform in hexane, 100 ml of 80 percent chloroform in hexane, 50 ml of 90 percent chloroform in hexane, 150 ml of chloroform, and 50 ml quantities of 2, 4, 6, 8 and 10 percent methanol in chloroform. The retention volume of the classes varied due to the variation in carbonyl content among cheese samples.

The column eluate was monitored for absorption at 254 nm with an Isco Model UA-2 ultra-violet analyzer (Instrumentation Specialities Co., Inc., Lincoln, Nebraska). A strip chart recording of absorption was made

and each peak was collected separately. The chromatographic fractions were evaporated to dryness under nitrogen, and the residues dissolved in chloroform. A Beckman DK-2A ratio recording spectrophotometer was used to establish class authenticity on the basis of the following absorption maxima: methyl ketones, 363; saturated aldehydes, 358; 2-3nal, 374 (Day, 1965).

Separation of Methyl Ketone DNPH into Individual Chain Lengths: The methyl ketone class was separated into its individual members using the liquid-liquid partition column of Corbin, Schwartz and Keeney (1960). Twenty-five grams of Celite, analytical filter aid (dried at 150° C for 24 hrs) were slurried with 250 ml hexane equilibrated with acetonitrile. Thirty-three ml acetonitrile and 0.5 ml distilled water were added and the blended slurry was poured into a 2.8 cm ID chromatographic column. The column was packed with nitrogen pressure (6-8 psi). Approximately 1000 ml of hexane equilibrated with acetonitrile was required to elute the ketones. The column effluent was monitored for absorption at 254 nm with an Isco Model UA-2 ultraviolet analyzer and a strip chart recording was made. The individual members of the ketone class were collected and the solvent evaporated under a stream of nitrogen on a steam bath.

Determination of Individual Methyl Ketone Concentration: The concentrations of the C₅-C₁₃ methyl ketone derivatives were determined by measuring their absorbance

in chloroform at 363 nm. The molar absorptivities of the methyl ketone 2,4 DNP derivatives used for quantitation were those reported by Day (1965). The chain length of each methyl ketone derivative was tentatively assigned by its retention volume on the liquid-liquid partition column.

Thin-Layer Chromatography of Methyl Ketones: Confirmation of chain length was made using the thin-layer chromatography methods of Libbey and Day (1964), and Edwards (1966). Prepoured plates of silica gel F-254 (Brinkman Instruments, Inc.) were used for both methods.

The method of Libbey and Day involved activating the plates at 150° C for one hour. After cooling, they were placed in a developing tank containing petroleum ether with 10% silicone oil for 5-6 hr. The ether was allowed to evaporate and the plates spotted with DNPH samples, then developed in dioxane-H₂O (65:35, v/v) for 6-8 hours.

The other method (Edwards, 1966) required a 24 hour plate activation. The plates were developed in hexane-ethyl acetate (4:1, v/v). To visualize the DNPH, the plates were sprayed with 0.2% 2,7-dichlorofluorescein in ethanol and then with 5N NaOH. Spots were visible as deep red-orange on an orange background.

Determination of Percentage Recovery of the Individual Methyl Ketones: A standard mixture of the C₅-C₁₃ methyl ketones was used to determine the percentage recovery. Concentrations used approximated those found in Blue cheese.

The standard ketone mixture in hexane was passed over a 2,4 DNP reaction column to convert the ketones to their hydrazones. Three grams of butteroil were added to the standard mixture and the sample analyzed as described for cheese. The fat was added after hydrazone formation to reduce the possibility of any methyl ketones present reacting to form derivatives. Fat added in this manner did not contribute any hydrazones to the sample.

All analyses were carried out in duplicate.

Production of Quick-Ripened Blue Cheese

Quick-ripened Blue cheese was made according to the method of Hedrick et al (1968). Raw milk containing 3.5 percent fat was preheated to 130° F and homogenized in a two stage homogenizer (2000 psi first stage, 500 psi second stage). Following homogenization the milk was pasteurized at 143° F for 30 minutes and then cooled to 84° F.

Cheese was made in 20 gallon batches using 17 g frozen concentrated cheese starter per batch. After the acidity had risen, .02%, 10 ml of decolorizer (chlorophyll and food dye in alkaline solution), 24 g mold powder ("Midwest" Blue Mold Powder) and 10 ml single strength rennet were added per 20 gallons of milk. When the curd separated cleanly (usually one hour), it was cut with 3/8" cheese knives. The curd was stirred thirty minutes after cutting and again thirty minutes later. After the second stirring,

the whey was drained and the curd placed on a stainless steel mesh screen in the ripening room. The depth of the cheese curd was approximately 3 to 4 inches.

The curing room was maintained at 62° F and 95% relative humidity. The curd was stirred daily and salt was added on the fourth, fifth and sixth days. The salting rate was one pound for each 22 pounds of cheese. One-fourth was added on the fourth day, one-fourth on the fifth day, and one-half on the sixth day. The ripened Blue cheese had a salt content of approximately 4%.

Subsequent batches of cheese were varied as to manufacture and ripening conditions (Table 1). After ripening 10 days the cheese usually had a flavor characteristic of Blue cheese and was packaged in polyethylene bags and immediately frozen at -5° F and held at this temperature for further analysis.

Preparation of White Mold Powder

A slant of a white mutant of P. roqueforti was obtained from Midwest Mold Company and propagated by the method of Hussong and Hammer (1935). Whole wheat bread was cut into 1/2" cubes and placed in wide mouth bottles. The bottles were sterilized at 15 lbs. steam pressure for 30 minutes. After cooling, the bread was inoculated with mold and incubated at 21° C. Approximately 11 days was required for spore formation. The material was then pulverized in a Waring blender and stored at 5° C.

Table 1

Variations of Quick-Ripened Blue Cheese Produced

Batch	Variation	Time	Temperature	Salting	Comments
A	Lactic Acid Direct Acidification	7 days	62° F	$\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$ on days 4, 5 and 6	Lactic Acid (2N) was added to a pH of 5.7. Rennet, decolorizer and mold powder were then added.
B	White Mold	10 days	52° F	$\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$ on days 4, 5, 6	Curd was cooked after cutting.*
C	Control	10 days	62° F	$\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$ on days 4, 5 and 6	Method of Hedrick et al (1968)
D	Control Stored for 2 Weeks at 40° F	10 days	62° F	$\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$ on days 4, 5 and 6	Method of Hedrick et al (1968)
E	Suggested Standard #1	11 days	52° F	$\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$ on days 4, 5 and 6	Curd was cooked after cutting.*
F	Suggested Standard #2	11 days	52° F	$\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$ on days 1, 9 and 10	Curd was cooked after cutting.*

* A large amount of cheese was lost as curd fines during the standard cheese making operation due to shattering. To help prevent this loss the cheese was heated to 100° F during a period of $\frac{1}{2}$ hour. This process produced a firmer curd, increased the yield of cheese, and reduced the amount of time required to drain the whey.

RESULTS AND DISCUSSION

The results of this study have been arranged in the following manner. For convenience in discussing this research the data are presented, followed by a discussion of the effects of each processing variable. All modifications of quick ripened Blue cheese were made in duplicate.

Sensory Evaluation of Quick Ripened Blue Cheese

Each modification of quick ripened Blue cheese was evaluated for color, flavor and texture by a panel of experienced judges. Flavor was assigned a maximum possible total of 20 points, color 15 points and texture 10 points. It was felt that flavor should be given maximum importance because the quick ripened cheese is suitable for incorporation into other products. Color and texture were considered to be of lesser importance. A cheese with a score of approximately 30 out of the total of 45 was considered acceptable. The commercial control was a packaged, crumbled Blue cheese purchased in a local supermarket.

Quantitation of Methyl Ketones

Sensory evaluation of quick ripened Blue cheese showed that most samples were lacking in methyl ketone flavor, a

flavor which is considered typical of good Blue cheese. During this study it was observed that the flavor score was directly related to methyl ketone content. Starkle (1924) and Hammer and Bryant (1937) postulated that methyl ketones contribute to the flavor and aroma of Blue mold cheeses. Hammer and Bryant isolated pentanone-2, heptanone-2 and nonanone-2, as did later workers (Patton, 1950; Morgan and Anderson, 1956). The quantity and distribution of methyl ketones in quick ripened Blue cheese was studied as it relates to processing parameters.

The methyl ketone content of the quick ripened cheeses studied in this project, reported as micromoles/10 g of extracted fat, are presented in Table 3. The processing variations have been outlined in Table 1 and described in detail in Experimental Methods. These samples were chosen for analysis out of a larger series of different quick ripened Blue cheeses which had been prepared in this laboratory (Harte, 1974; Kuehler, 1974). This selection of samples for analyses was necessary due to the length of time required for complete separation and identification of the methyl ketones in a single sample (approximately two weeks per sample). With one exception, the samples were chosen to represent the best of the cheese prepared in our laboratory. One poor quality cheese (that prepared by direct acidification) was also analyzed. The methyl ketone content paralleled the sensory evaluations made on the cheeses.

Table 2

Sensory Evaluation of Quick Ripened Blue Cheese

Cheese	Flavor	Body & Texture	Color	Total
Lactic Acid, Direct Acidification	7.3	5.0	4.5	16.8
White Mold	9.7	7.3	11.0	28.0
Control	12.0	6.3	11.6	29.9
Control, Stored for 2 Weeks at 40° F	18.0	8.0	11.6	37.6
Suggested Standard #1	15.6	7.0	12.5	35.0
Suggested Standard #2	14.0	8.0	12.0	34.0
Commercial Cheese	18.0	7.0	14.8	38.0

Table 3

Concentration of Methyl Ketones in Fat Extracted From Blue Cheese
(micromoles/10 g of Extracted Fat)

Methyl Ketone Chain Length	Cheese samples ¹					
	Commercial			Quick ripened ²		
	A	B	C	D	E	F
5	17.13	trace	.41	4.12	2.97	4.38
7	63.40	12.43	20.48	21.56	49.28	56.74
9	86.45	12.57	38.74	53.96	54.58	65.10
11	22.37	2.36	9.37	1.58	3.90	7.54
13	3.44	1.19	.70	trace	.99	1.71
Total	192.79	28.55	64.87	81.22	111.72	135.47

¹Average of duplicate analyses.

²Quick ripened Blue cheese samples:

A Lactic Acid, Direct Acidification

B White Mold

C Control

D Control Stored for 2 Weeks at 40° F

E Suggested Standard #1

F Suggested Standard #2

All samples contained large amounts of heptanone-2 and nonanone-2. Dartey and Kinsella (1971) observed that these two methyl ketones accounted for 50 to 75% of the total methyl ketone in Blue cheese. As shown by Blakely (1970), nonanone-2 was found to be the major methyl ketone in quick ripened Blue cheese. In addition to those ketones reported, small amounts of a compound assumed to be C₁₅ methyl ketone (pentadecanone-2) were found in all samples analyzed. The presence of C₁₃ and C₁₅ methyl ketones in Blue cheese may be due to natural breakdown of milk fat, rather than to microbial action (Schwartz and Parks, 1963). Results of these analyses are comparable to the findings of Morgan and Anderson (1956), Schwartz and Parks (1963), and Anderson and Day (1966), in that the individual ketones in Blue cheese vary considerably, even among cheeses of good quality.

Quantitation of Free Fatty Acids

These data were collected for quick ripened Blue cheese by Harte (1974), and are presented in this thesis in Table 4. The cheese samples analyzed are identical to the samples used in this study and are included here for discussion because of their interrelation with methyl ketone data and flavor properties. Free fatty acids (FFA) were determined by the method of Iyer, Richardson, Amundson and Boudreau (1967). Table 4 shows the FFA content of the quick ripened Blue cheese, reported as mg acid/Kg cheese.

Table 4

Concentration of Free Fatty Acids In
Quick Ripened Blue Cheese (mg/Kg).¹

ACID	Cheese sample ²					
	A	B	C	D	E	F
Butyric	846	657	1,270	1,680	650	875
Caproic	465	228	490	485	244	401
Caprylic	523	645	424	359	314	306
Capric	2,010	1,460	824	2,240	950	2,350
Lauric	2,580	2,380	1,240	1,500	1,540	2,560
Myristic	6,720	4,630	4,090	4,660	4,630	4,520
Palmitic	9,780	10,200	12,500	12,500	13,100	12,400
Stearic	4,370	4,910	6,590	6,460	4,730	5,600
Oleic	10,930	11,100	13,900	13,000	12,900	13,000
Linoleic	1,060	936	787	750	934	1,260
Linolenic	862	844	707	651	762	914

¹From Harte, 1974.

²Quick ripened Blue cheese samples:

- A Lactic Acid, Direct Acidification
- B White Mold
- C Control
- D Control Stored for 2 Weeks at 49° F
- E Suggested Standard #1
- F Suggested Standard #2

Fatty acids are metabolized by the mold spore to form β -keto acids and then decarboxylated to form methyl ketones of one less carbon atom (Hawke, 1966).

Therefore, butyric through myristic acids are oxidized to form the C_5 through C_{13} methyl ketones quantitated in this study. The data for the longer chain acids are also included in Table 4 because of their contribution to the flavor of Blue cheese. Anderson and Day (1965) and others felt that the long chain fatty acids may exist in the salt form, accounting for the slightly "soapy" flavor characteristic of some mature Blue cheeses.

Comparison of the fatty acid and methyl ketone data does not reveal any particular relationship of fatty acid concentration to ultimate levels of methyl ketone. For example, in samples C and D (a control quick ripened cheese and the same cheese after two additional weeks storage at 40° F) the caproic acid contents are 490 and 485 mg/Kg respectively. However there is a ten fold increase in the corresponding methyl ketone, 2-pentanone, in D, the stored sample, indicating a highly significant conversion of fatty acid to ketone. The capric (C_{10}) acid content of the stored control increased three fold over the initial value and the corresponding ketone, 2-nonanone, showed a 50% increase during storage. Thus, from these data, one cannot predict the levels of methyl ketone in a sample of cheese and certainly not from FFA data. Naturally, the relative concentration of the two classes of

compounds are quite different, the ketones being reported in micromoles/10 g fat and the FFA as mg/Kg cheeses. If there is some limiting factor governing the rate of conversion of FFA to methyl ketone one could observed an increase in total FFA without a concomitant increase in ketone.

The amount of methyl ketone produced may be related to the amount of fatty acid available. The results of Girolami and Knight (1955) show that the formation of ketones is directly dependent on the quantity of available fatty acid. However, later work (Anderson, 1966) showed that the amount of ketone produced is also related to the chain length of the corresponding fatty acid. Octanoic acid was found to convert to methyl ketone fastest, while butanoic and dodecanoic acids were the slowest.

Methyl Ketone Content of a Commercial Blue Cheese Sample

A sample of commercial Blue cheese packed in loose crumbled form was analyzed for methyl ketones. This cheese was judged by sensory evaluation to be slightly superior (Table 2) to all samples of quick ripened cheese and the high quantities of ketone (Table 3) confirm this. Apparently the flavor score is related to net ketone levels in the concentrations shown here. The commercial sample was analyzed for the purpose of comparison with the quick ripened Blue cheeses. The results show a similar ketone distribution, but a much higher overall quantity in the

commercial cheese. The quick ripened samples ranged from 28.55 micromoles/10g fat for cheese made by direct acidification with lactic acid to 135.47 micromoles for cheese ripened at 52° F and salted on days 1, 9 and 10. This is in contrast to 192.79 total micromoles for the commercial cheese.

Nonanone-2 was the most abundant ketone in this sample, as in the quick ripened samples. Anderson and Day (1966) found 2-heptanone to be the predominant ketone in two samples of Roquefort and five domestic Blue cheese samples. Two of the domestic Blue cheese samples contained equivalent concentrations of 2-nonanone and 2-heptanone. In three samples of domestic Blue cheese, Schwartz and Parks (1963) observed 2-heptanone to be the most abundant ketone. A Roquefort sample analyzed by Schwartz, Parks and Boyd (1963) contained more 2-nonanone than 2-heptanone. Blakely (1970) found 2-nonanone to be the major methyl ketone in samples of quick ripened Blue cheese.

Quick Ripened Blue Cheese Made by Direct Acidification with Lactic Acid

One of the more time consuming steps in the manufacture of quick ripened Blue cheese is the ripening of the cheese milk with starter culture. Approximately 1 to 1 1/2 hours are required for the acidity to rise .02%. In an attempt to shorten the procedure, quick ripened Blue cheese was made by direct acidification with lactic acid, without the

use of starter culture. Lactic acid was added to the cheese milk until the pH had been brought down to 5.70. This was close to the pH of cheese milk ripened with starter culture.

A Blue cheese possessing good body and texture was produced by a direct acidification method by Shehata and Olsen (1967). These workers obtained better results using hydrochloric acid instead of lactic acid. They found the lactic acid curd too soft and too high in moisture. Singh and Kristofferson (1972) attempted to produce a Blue cheese flavor using a slurry prepared from directly acidified lactic acid curd. The curd had physical characteristics comparable to normal Blue cheese, but it failed to develop characteristic flavor during storage. Shehata (1967) incorporated a lipolytic enzyme into milk used for directly acidified cheese in order to encourage the development of flavor compounds.

Quick ripened Blue cheese made in our laboratory by this method of direct acidification failed to develop a flavor characteristic of good Blue cheese. This observation was verified by the much lower methyl ketone content of the directly acidified cheese compared to other quick ripened Blue cheeses.

As shown in Table 3, the total concentration of methyl ketones was 28.55 micromoles/10g of fat compared to 64.87 for the control cheese. The levels of heptanone-2 and nonanone-2 were almost equal and together they accounted

for greater than 85% of the total methyl ketones. The other ketones are almost negligible in concentration. This unusual ketone distribution may in part account for the atypical flavor in this cheese sample.

The directly acidified quick ripened cheese also failed to exhibit the characteristic drop in pH after manufacture (Coulter, 1938b). Conventional Blue cheese reaches a minimum pH of 4.7 within one day after manufacture. This increase in acidity was also characteristic of quick ripened Blue cheese (Harte, 1974). Most quick ripened Blue cheese samples reached a maximum acidity on day 2 or 3. However, cheese made by this method required four days to reach its minimum pH. Also, the pH did not drop as far as it did with other variations. The directly acidified curd reached a pH of 5.37, while the pH of standard quick ripened Blue cheese fell as low as pH 4.86 (Harte, 1974). This slow, atypical change in pH may be indicative of an abnormal mold growth and consequent poor flavor production.

This cheese received the lowest rating by sensory evaluation since it failed to develop characteristic flavor and color. Using the processing conditions described, this cheese was unacceptable. However, longer ripening might have improved the cheese.

Quick Ripened Blue Cheese Made Using a Mutant White Mold

Cheese was made using a white mutant strain of P. roqueforti. The standard method of Hedrick et al (1968) was used, but the ripening temperature was lowered to 52° F and the curd was cooked to 100° F after cutting. The white mutant strain was developed by Knight et al (1950) by the irradiation of spores. The intent of using a white mutant in cheesemaking is to produce a cheese possessing a typical Blue cheese flavor without the color of the mold being evident. This would be preferable in some applications and could conceivably increase consumer acceptance of Blue cheese products.

The total concentration of methyl ketones in the white cheese was 41.38 micromoles/10 g fat (Table 3). This was fairly low compared to other samples analyzed in this study, but still slightly higher than the values reported by Blakely (1970) for quick ripened Blue cheese. Blakely's values ranged from 17.7-26.0 micromoles/10 g fat. The total FFA as outlined in Table 4 for this cheese were also low compared to other samples of quick ripened Blue cheese (Harte, 1974). The low ketone content is in contrast to the results of Nelson (1970). Nelson quantitated the total ketones in a submerged culture of white mutants of P. roqueforti grown in homogenized milk plus lipolyzed milk fat. He detected a ketone concentration 7 to 15 times that found in Blue cheese; however his

results were obtained in a liquid culture rather than a ripened cheese.

The ketone profile of quick ripened Blue cheese made with white mold was fairly similar to that of conventional Blue cheese and other quick ripened Blue cheese samples. The only major difference was the substantially lower concentration of 2-heptanone (Table 4). The caprylate level was also low as determined by Harte (1974). This may reflect a difference in the lipase system of the mutant white mold.

The white cheese was rated by sensory evaluation as having a mild flavor, and again the low intensity of flavor is reflected in a low concentration of methyl ketones. This may be due to the fact that the ripening temperature was only 52° F, yet the salting was done on days 4, 5 and 6. With conventional quick ripened Blue cheese salting times were determined by the development of the blue color. This of course was not possible with the white mutant. Harte (1974) and Kuehler (1974) have shown that early salting may slow down mold activity and consequently the production of flavor compounds. Knight et al (1950) claimed Blue cheese manufactured by a traditional method using the white mutant had normal lipolytic activity, but a milder flavor and softer body than normal Blue cheese. The level of flavor compounds may have been higher had salting been delayed. Additonal research is needed in this area.

Quick Ripened Blue Cheese Made by the Original Method and
Stored at 40° F for 2 Weeks

A control batch of cheese was made by the original method of Hedrick et al (1968). The cheese was ripened at 62° F for 7 days and salted on days 4, 5 and 6. Some of this cheese was then stored for 2 weeks at 40° F. The stored cheese was also analyzed for methyl ketones. Sensory evaluation of both cheeses showed the stored cheese to have a much finer flavor, although the mold color was the same.

Both cheeses had high methyl ketone levels. The data in Table 3 show that the total methyl ketone content of the original cheese was 64.87 micromoles/10 g of fat, and the stored cheese was approximately 16 micromoles higher. The level of 2-nonanone showed the most marked increase during storage. Undecanone-2 actually decreased in concentration during storage, possibly due to interconversion with the corresponding secondary alcohol. The ketone level in this standard batch of cheese was significantly higher than that reported by Blakely (1970) for quick ripened Blue cheese.

The FFA data (Table 4) show that the volatile (C₄-C₁₀) fatty acids increased in storage. These data, and the increase in methyl ketones discussed above indicate that the enzyme systems of P. roqueforti are active during storage at 40° F. A cheese of quality superior to the original standard cheese was produced by 2 weeks storage.

Suggested Methods of Manufacture for Quick Ripened Blue Cheese

Many variations on the original method of Hedrick et al (1968) were attempted in an effort to improve the quick ripened Blue cheese. It was felt that more control was needed over the fermentation in order to provide better, more easily reproducible results. The two variations discussed in this section are suggested as an improved method of manufacture.

Several problems were encountered during the production of the standard batch discussed above. Contamination by microorganisms of the ripening curd was often seen. Better control of the salting procedure and a lower ripening temperature were tried in an effort to eliminate or at least minimize this problem. Also, in an effort to cut down the drainage time, produce a firmer curd, and reduce curd shattering, the cheese was cooked to 100° F. A ripening temperature of 52° F was used. One batch was salted on days 7, 8 and 9, the other on days 1, 9 and 10. Both were allowed to ripen for 11 days.

Cooking the cheese curd was considered preferable to the original method. Less shattering occurred and therefore less loss of curd fines was noted during drainage. This was verified by weighing curd made by each method. Also, the drainage time required was greatly reduced when cooking was introduced, thereby reducing the amount of labor required in the production of the cheese.

A lower ripening temperature was also considered to be preferable to the original temperature of 62° F. Morris (1969) demonstrated that higher temperatures are superior for increased lipolysis and proteolysis, but the body, color and flavor of the cheese are of lower quality. As indicated in Table 2, subjective analysis of cheese made by the standard method and by the two suggested methods showed high scores in all areas for the suggested methods compared to other quick ripened Blue cheeses. The increased flavor intensity may be due to the longer ripening time. Also, this longer ripening time may have provided for a better balance of flavor components. The methyl ketone content of the cheese made by the suggested methods was approximately double that of the standard method (Table 3).

The methyl ketone content of cheese made by the second suggested method (salting on days 1, 9 and 10) was slightly higher, although both cheeses were highly acceptable. This variation and control of salting was felt to be extremely important in control of flavor development (Harte, 1974; Kuehler, 1974). Most organisms are inhibited at the 10 percent brine concentrations present in Blue cheese. The control of contamination in quick ripened Blue cheese is essential because of the rapid growth of organisms on the large area of curd surface exposed to the atmosphere.

The enzyme systems of P. roqueforti are also affected by salt concentration. Willart and Sjostrom (1959), using

salt concentrations of 1.5-4.5%, found less higher chain length fatty acids at the higher levels of salt. Morris and Jezeski (1953) and Inamura (1960) reported that the lipolytic activity of P. roqueforti decreased with increasing salt concentration.

The time of salting and amount of salt were used to control mold growth and activity in quick ripened Blue cheese. According to the data of Harte (1974), the addition of salt on day 1 caused a delay in the normal drop in pH. This may be due to inhibition of the starter culture by the salt and therefore a slower production of lactic acid. P. roqueforti may also be inhibited by an early addition of NaCl. Harte (1974) found that lipolysis in quick ripened cheese salted on days 1, 4 and 5 was retarded. However, cheese salted on days 1, 9 and 10 showed normal lipolytic activity as evidenced by the methyl ketone and FFA data (Tables 3 and 4). The difference here is the time span between the initial and final saltings. This longer time interval may have allowed the enzyme systems of P. roqueforti to overcome the initial slow start. The early salting may even have been beneficial for carbonyl production. Nelson (1970) observed that carbonyl production by spores of P. roqueforti is enhanced by the addition of salt. However, a lag phase caused by salt addition was observed by later workers (Dwivedi and Kinsella, 1974). These researchers noticed a prolonged lag phase before carbonyl production by mycelium when salt

was added to the growth media. The salting times suggested here (days 1, 9 and 10) may have been ideal for carbonyl production by allowing time for the mold to overcome the lag phase, yet enhancing total carbonyl production by early salting. The cheese made by this second method was described as having a clean, milk flavor. It is suggested that salting on day 1 to control contaminating organisms is a satisfactory method of production. No problems with contamination were encountered using this procedure.

SUMMARY AND CONCLUSIONS

Quick ripened Blue cheese was prepared according to the method of Hedrick et al (1968). This original procedure was varied in an attempt to standardize and improve the cheese. The following modifications were developed and studied:

1. Direct acidification with lactic acid
2. Use of a mutant white strain of P. roqueforti
3. Cooking curd to 100° F during the processing of the cheese
4. Ripening at 52° F with salting on days 1, 9 and 10
5. Ripening at 52° F with salting on days 7, 8 and 9
6. Storage of the quick ripened cheese at 40° F for 2 weeks to improve flavor quality.

All samples were subjected to sensory evaluation. The methyl ketone content of the cheese was determined by chromatographic separation and quantitation of 2, 4 dinitro-phenylhydrazine derivatives of the C₅-C₁₅ carbonyls. The cheeses were compared using a conventionally ripened commercial Blue cheese as a control.

The commercial sample was judged superior to all samples of quick ripened Blue cheese. Nonanone-2 was the most abundant ketone in all of the samples analyzed.

Directly acidifying the curd with lactic acid produced a quick ripened Blue cheese of poor quality. The methyl ketone content was extremely low and the flavor was atypical. The pH of such cheese during ripening was considered abnormal when compared to other quick ripened cheeses. Due to the lack of characteristic flavor and color, this cheese received the lowest rating by sensory evaluation.

Cheese made with a white mutant strain of P. roqueforti was considered satisfactory, although low in flavor and methyl ketone content. The flavor was typical of a good, milk Blue cheese although the typical blue-green color of the mold was absent. This cheese was considered excellent for incorporation into dips and dressings where lack of mold color is desirable.

Quick ripened Blue cheese was shown to be improved by storage of the ripened curd at 40° F. Cheese stored for two weeks was judged superior in flavor. The total methyl ketone content was also markedly increased; nonanone-2 increased to the greatest extent, while undecanone-2 decreased in storage.

Two modified methods of manufacture of quick ripened cheese were developed. These modifications resulted in improved flavor, body and texture of the cheeses. The methyl ketone content of the cheese prepared by either modified method was significantly higher than conventional

quick ripened cheeses. These variations involved cooking of the cut cheese curd, lowering the ripening temperature to 52° F, and salting either on days 1, 9 and 10 or on days 7, 8 and 9.

REFERENCES

- Albrecht, T. W. and H. O. Jaynes. 1955. Milk lipase. J. Dairy Sci. 38:137.
- Anderson, D. F. 1966. Flavor chemistry of Blue cheese. Ph.D. Thesis. Oregon State University, 95 pp.
- Anderson, D. F. and E. A. Day. 1965. Quantitative analysis of the major free fatty acids in Blue cheese. Ph.D. Thesis. Pennsylvania State College, 37 pp.
- Anderson, D. F. and E. A. Day. 1966. Quantitation, evaluation, and effect of certain microorganisms on flavor components of Blue cheese. J. Agri. Food Chem. 14:241.
- Association of Official Agricultural Chemists (AOAC). 1970. Official Methods of Analysis. Eleventh Edition. Publ. by AOAC, Washington, D.C.
- Bassett, E. W. and W. J. Harper. 1958. Isolation of identification of acids and neutral carbonyl compounds in different varieties of cheese. J. Dairy Sci. 41:1206.
- Bavisotto, U. S., L. A. Rock and R. S. Lesniewski. 1960. Gas chromatography of volatiles of fermented milk products. J. Dairy Sci. 43:849.
- Blakely, L. E. 1970. A study of the formation of methyl ketones and free fatty acids in quick ripened Blue cheese and subsequent losses during dehydration. Ph.D. Thesis. Michigan State University, 180 pp.
- Boyd, E. N., P. G. Keeney and S. Patton. 1965. The measurement of monocarbonyl classes in cocoa beans and chocolate liquor with special reference to flavor. J. Food Sci. 30:854.
- Chanet, J. 1972. Improvements in cheese making. French patent 2 104 979. Food Sci. Tech. Abstr. 2P:150.

- Coffman, J. R., D. E. Smith and J. S. Andrews. 1960. Analysis of volatile food flavors by gas-liquid chromatography. I. The volatile components from dry Blue cheese and dry Romano cheese. *Food Res.* 25:663.
- Corbin, E. A., D. P. Schwartz and M. Keeney. 1960. Liquid-liquid partition chromatography. Separation of the 2,4-dinitrophenylhydrazones of saturated aldehydes, methyl ketones, 2-enals and 2,4-dienals. *J. Chromatog.* 3:322.
- Cort, W. M. and L. K. Riggs. 1967. Enzymatic process of making cheese and cheese products. U. S. Patent 3 295 991.
- Coulter, S. T., W. B. Combs and J. S. George. 1938a. The influence of acidity variations during manufacture on the quality and rate of ripening of Blue or American Roquefort cheese. *J. Dairy Sci.* 21:239.
- Coulter, S. T., W. B. Combs and J. S. George. 1938b. The pH of Blue or American Roquefort cheese. *J. Dairy Sci.* 21:273.
- Currie, J. N. 1914. Flavor of Roquefort cheese. *J. Agri. Res.* 2:1.
- Dartey, C. K. and J. E. Kinsella. 1971. Rate of formation of methyl ketones during Blue cheese ripening. *J. Agri. Food Chem.* 19:771.
- Dartey, C. K. and J. E. Kinsella. 1973. Oxidation of sodium ($U^{14}C$) palmitate into carbonyl compounds by *P. roqueforti* spores. *J. Agr. Food Chem.* 21:721.
- Day, E. A. 1965. Some properties of carbonyl compounds encountered in the flavor isolates of dairy products--a review. *Food Technol.* 19:1585.
- Day, E. A. and D. F. Anderson. 1965. Gas chromatographic and mass spectral identification of natural components of the aroma fraction of Blue cheese. *J. Agri. Food Chem.* 13:2.
- Dwivedi, B. K. and J. E. Kinsella. 1974. Carbonyl production from lipolyzed milk fat by the continuous mycelial culture of *Penicillium roqueforti*. *J. Food Sci.* 39:83.
- Eitenmiller, R. R., J. R. Vakil and K. M. Shahani. 1970. Production and properties of a *Penicillium roqueforti* lipase. *J. Food Sci.* 35:130.

- Edwards, H. M., Jr. 1966. Thin-layer chromatography of stereoisomeric 2,4-dinitrophenylhydrazones of aliphatic aldehydes. *J. Chromatog.* 22:29.
- Fryer, T. F. and B. Reiter. 1967. Lipolytic activity of lactic acid bacteria. *J. Dairy Sci.* 50:388.
- Gehrig, R. F. and S. G. Knight. 1958. Formation of ketones from fatty acids by spores of Penicillium roqueforti. *Nature* 182:1237.
- Gehrig, R. F. and S. G. Knight. 1963. Fatty acid oxidation by spores of Penicillium roqueforti. *Applied Microbiol.* 11:166.
- Girolami, R. L. and S. G. Knight. 1955. Fatty acid oxidation by Penicillium roqueforti. *Applied Microbiol.* 3:264.
- Gould, I. A. 1941. Effect of certain factors upon lipolysis in homogenized raw milk and cream. *J. Dairy Sci.* 24:779.
- Hammer, B. W. and H. W. Bryant. 1937. A flavor constituent of Blue cheese (Roquefort type). Iowa State College. *J. Sci.* 11:281.
- Harper, W. J. 1959. Chemistry of cheese flavors. *J. Dairy Sci.* 42:207.
- Harper, W. J. and P. Gaffney. 1970. Lipase. I. Characterization of the milk lipase system. Centennial Res. Ser. Dep. Dairy Tech. Ohio State University. 6:1.
- Harper, W. J. and T. Kristofferson. 1956. Biochemical aspects of cheese ripening. *J. Dairy Sci.* 39:1773.
- Harte, B. R. 1974. The effects of process parameters on the formation of volatile acids and free fatty acids in quick ripened Blue cheese. M.S. thesis. Michigan State University, 102 pp.
- Harwalker, V. R. and H. E. Calbert. 1961. Specificity of milk lipases. *Abstr. J. Dairy Sci.* 44:1169.
- Hawke, J. C. 1966. Reviews of the progress of dairy science. Section D. Dairy chemistry. The formation and metabolism of methyl ketones and related compounds. *J. Dairy Res.* 33:225.

- Hedrick, T. I., E. Kondrup and W. T. Williamson. 1968. Preparation of Blue cheese by adding to the cheese a Blue cheese mold and ripening the curd in a divided condition. U. S. Pat. 3, 365,303.
- Hornstein, I. and P. F. Crowe. 1962. Carbonyl removal from non-oxygenated solvents. Anal. Chem. 34:1037.
- Hussong, R. V. and B. W. Hammer. 1935. The preparation of mold powder for blue-veined cheeses. J. Dairy Sci. 18:599.
- Imamura, T. and K. Kataoka. 1963b. Biochemical studies on the manufacturing of Roquefort type cheese. II. Characteristics of lipases produced by Penicillium roqueforti. Jap. J. Zootech. Sci. 34:349 in Dairy Sci. Abstr. 26:1696. 1964.
- Imamura, T. and K. Kataoka. 1966. Biochemical studies on the manufacturing of Roquefort type cheese. III. Isolation of lipases from mold-culture. Jap. J. Dairy Sci. 15:A138. in Dairy Sci. Abstr. 29:1525. 1967.
- Iyer, Mrs. Meena, T. Richardson, C. H. Amundson and A. Boudreau. 1967a. Improved technique for analysis of free fatty acids in butteroil and Provolone cheese. J. Dairy Sci. 50:285.
- Jackson, H. W. and R. V. Hussong. 1958. Secondary alcohols in Blue cheese and their relation to methyl ketones. J. Dairy Sci. 41:920.
- Jensen, R. G. 1964. Lipolysis. J. Dairy Sci. 47:210.
- Knight, S. G., W. H. Mohn and W. C. Frazier. 1950. White mutants of Penicillium roqueforti. J. Dairy Sci. 33:929.
- Kondrup, E. and T. I. Hedrick. 1963. Short time curing of Blue cheese. Michigan State Agri. Expt. Sta. Quart. Bull. 46:156.
- Kuehler, C. K. 1974. The effects of process parameters on the flavor and protein degradation in quick ripened Blue cheese. Ph.D. thesis. Michigan State University, 174 pp.
- Lane, C. B. and B. W. Hammer. 1938. Bacteriology of cheese. III. Some factors affecting the ripening of Blue (Roquefort type) cheese. Iowa Agri. Expt. Sta. Res. Bull. 237:197.

- Lawrence, R. C. 1966. The oxidation of fatty acids by spores of Penicillium roqueforti. J. Gen. Microbiol. 44:393.
- Lawrence, R. C. and J. C. Hawke. 1968. The oxidation of fatty acids by mycelium of Penicillium roqueforti. J. Gen. Microbiol. 51:289.
- Lundstedt, E. 1973. Preparation of Blue cheese from soybean milk. U. S. Pat. 3,743,515.
- Magidman, P., S. F. Herb, R. A. Barford and R. W. Riemschneider. 1962. Fatty acids of cows' milk. A. Techniques employed in supplementing gas-liquid chromatography for identification of fatty acids. J. Am. Oil Chemist Soc. 39:137.
- Matheson, K. J. 1921. Manufacture of cows' milk Roquefort cheese. U.S.D.A. Bull. 970.
- Modler, H. W., J. R. Brunner and C. M. Stine. 1974. Extracellular protease of Penicillium roqueforti. II. Characterization of a purified enzyme preparation. J. Dairy Sci. 57:528.
- Morgan, M. E. and E. O. Anderson. 1956. The neutral carbonyl compounds in Blue-mold type cheese. J. Dairy Sci. 39:253.
- Morris, T. A. 1963. The effect of draining Blue-vein cheese curd prior to hooping. Austr. J. Dairy Technol. 18:195.
- Morris, T. A. 1969. Effect of ripening temperature on the properties of Blue-vein cheese. Aust. J. Dairy Technol. 24:9.
- Morris, H. A., W. B. Combs and S. T. Coulter. 1951. The relation of surface growth to the ripening of Minnesota Blue cheese. J. Dairy Sci. 34:209.
- Morris, H. A., J. J. Jezeski, W. B. Combs and S. Kuramoto. 1955. Free fatty acids produced during Blue cheese ripening. Abstr. J. Dairy Sci. 38:590.
- Morris, H. A., J. J. Jezeski, W. B. Combs and S. Kuramoto. 1963. Free fatty acid, tyrosine, and pH changes during ripening of Blue cheese made from variously treated milks. J. Dairy Sci. 46:1.
- Nawar, W. W. and I. S. Fagerson, 1962. Direct chromatographic analysis as an objective method of flavor measurement. Food Technol. 16:107.

- Nelson, J. H. 1970. Blue cheese flavor by fermentation. Food Prod. Dev. 4(1):52.
- Nesbitt, J. M. 1953. The formation of methyl ketones during the ripening of Blue cheese. Ph.D. thesis. Pennsylvania State College, 37 pp.
- Niki, T., Y. Yoshioka and K. Ahiko. 1966. Proteolytic and lipolytic activities of Penicillium roqueforti isolated from Blue cheese. XVII. Int. Dairy Cong. D:531.
- Nishikwa, I. 1958. Studies on the proteolytic decomposition in cheese. II. Characteristics of the proteolytic enzyme of Penicillium roqueforti. Report Research Lab. Snow Brand Milk Prod. Japan. No. 36 in Dairy Sci. Abstr. 20:2887. 1958.
- Parmelee, C. E. and F. E. Nelson. 1949a. The use of a mold enzyme preparation in the manufacture of Blue cheese from pasteurized homogenized milk. J. Dairy Sci. 32:1001.
- Parmelee, C. E. and F. E. Nelson. 1949b. The relation of chemical analysis to flavor scores of Blue cheese made from pasteurized homogenized milk. J. Dairy Sci. 32:1007.
- Patton, S. 1950. The methyl ketones of Blue cheese and their relation to its flavor. J. Dairy Sci. 33:680.
- Patton, S. 1951. New roquefort type flavors. Food Engr. 12:93.
- Peters, I. I. and F. E. Nelson. 1961. Manufacture of Blue cheese. Milk Prod. J. 52(1):10.
- Poznanski, S., J. Jaworski and T. D'Obyrn. 1967. Characterization of products of fat hydrolysis in the ripening of Roquefort type cheese in relation to NaCl content. Dairy Sci. Abstr. 29:1666.
- Salvadori, P. and B. B. Salvadori. 1967. Preliminary observations on the lipolysis of some long chain fatty acids by different strains of Penicillium roqueforti. Lait. 47:605. in Dairy Sci. Abstr. 30:957. 1968.
- Sato, M., T. Honda, Y. Yamada, A. Takada and T. Kawanami. 1966. A study of free fatty acids, volatile carbonyl compounds and tyrosine in Blue cheese. XVII. Int. Dairy Congr. D:539.

- Schwartz, D. P., H. S. Haller and M. Keeney. 1963. Direct quantitative isolation of monocarbonyl compounds from fats and oils. *Anal. Chem.* 33:1396.
- Schwartz, D. P. and O. W. Parks. 1961. Preparation of carbonyl-free solvents. *Anal. Chem.* 33:1396.
- Schwartz, D. P. and O. W. Parks. 1963. Quantitative analysis of methyl ketones in Blue cheese fat. *J. Dairy Sci.* 46:989.
- Shehata, A. E. 1967. Manufacture of Blue cheese by direct acidification methods. Ph.D. Thesis. University of Wisconsin. in *Diss. Abstr.* 28:401.
- Shehata, A. E. and N. F. Olson. 1966. Manufacture of Blue cheese by direct acidification methods. *J. Dairy Sci.* 49:1025.
- Singh, S. and T. Kristofferson. 1972. Cheese flavor development using direct acidified curd. *J. Dairy Sci.* 55:744.
- Stadhoudes, J. and H. Mulder. 1957. Fat hydrolysis and cheese flavor. I. The enzymes responsible for the hydrolysis of fat in cheese. *Netherlands Milk and Dairy J.* II:164.
- Starkle, M. 1924. Methyl ketones in oxidative decomposition of some triglycerides (also fatty acids) by molds with regard particularly to the rancidity of cocoa fat. I. The significance of methyl ketones in the biochemistry of butter rancidity. (In German, English translation). *Biochemische Zeitschrift* 151:371.
- Stokoe, W. N. 1928. The rancidity of coconut oil produced by mold action. *Biochemical J.* 22:80.
- Tarassuk, N. P. and E. N. Frankel. 1957. The specificity of milk lipase. IV. Partition of the lipase system in milk. *J. Dairy Sci.* 40:418.
- Thom, C. and K. J. Matheson. 1914. Biology of Roquefort Cheese. *Conn. (Storrs) Agr. Expt. Bull.* 79:335.
- Thomasaw, J. Von. 1947. Secondary breakdown products of Blue-mold cheese. *Milchwissenschaft.* 2:354.
- Wilcox, J. C., W. O. Nelson and W. A. Wood. 1955. The selective release of volatile fatty acids from butter fat by microbial lipases. *J. Dairy Sci.* 38:775.

Willart, S. and G. Sjostrom. 1959. The influence of sodium chloride upon the hydrolysis of milk fat in milk and cheese. Int. Dairy Congr. 3:1480.

APPENDIX

Fat contents of cheese samples were routinely determined and are presented below.

Table 5
Fat Content of Blue Cheese Samples

Cheese Sample	% Fat ¹
Lactic Acid, Direct Acidification	29.72
White Mold	28.05
Control	26.83
Control, Stored for 2 Weeks at 40° F	25.38
Suggested Standard #1	26.61
Suggested Standard #2	27.76
Commercial Cheese	29.70

¹Average of duplicate analyses.

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