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QUICK RIPENING OF BLUE CHEESE CURD USING

CONTROLLED ATMOSPHERE PACKAGING

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Bruce Hartle

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QUICK-RIPENING OF BLUE CHEESE CURD USING CONTROLLED ATMOSPHERE PACKAGING

By

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ABSTRACT

QUICK RIPENING OF BLUE CHEESE CURD USING CONTROLLED ATMOSPHERE PACKAGING

By

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A Blue cheese of desirable quality was produced by a modification of the quick ripening (QR) procedure of Hedrick et al. (1968). The research described herein was designed to investigate the feasibility of quick ripening Blue cheese curd by selecting and maintaining the optimum atmosphere over packaged curd. Variables introduced included salting, temperature of ripening and modified and gas flushed atmosphere packaging. The use of physical methods of preservation such as ionizing radiation and microwave heating to control the accelerated ripening was investigated. To determine package permeability needed, the O_2/CO_2 requirement of the curd at various temperatures was investigated. During ripening of the cheese , pH, water-soluble nitrogen, gas headspace concentration, moisture content, and methyl ketone concentrations were determined. Sensory evaluation was also carried out on all batches of cheese.

The rate and magnitude of respiration were directly related to the ripening temperature. The CO_2 production ranged from 52.45 to 130.70 cc/kg/hr. A mutual dependent relationship existed between respiration and permeation within the Blue cheese packaging system.

As the pH values increased there was a corresponding increase in water-soluble nitrogen. The values for pH and water-soluble nitrogen at the end of ripening were 5.46-6.49 and 32 - 43%, respectively, depending on the ripening temperatures and the packaging procedures used.

The initial moisture content of Blue cheeses was 50.34% and decreased to 41.21-46.65% depending on the temperature, salt content and water vapor transmission of the package materials.

The predominant methyl ketone in QR Blue cheese curd was 2-heptanone, 2-pentanone and 2-nonanone were also present in substantial levels.

In cheeses irradiated with 1.0 KGy, a visible reduction of mold pigmentation and a slight decrease in methyl ketone concentrations were observed. A slight change of flavor, which was not objectionable, was also noted.

Microwave heating the cheese to 53.0°C and above was effective in reducing mold growth, however these

temperatures were detrimental to the flavor and texture of the cheese.

The cheeses ripened in modified atmosphere packaging were comparable or superior, as indicated by sensory evaluation, to commercial Blue cheese when an extended period of two weeks storage at 5°C was applied to the QR cheese.

Dedicated To
My Wife Safaa
My Daughter Sarah and My Son Yassir

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INTRODUCTION

The market for Blue mold cheeses and their related food products, salad dressings, chip dips and snack food has increased tremendously in recent years. Because of the high production costs and labor requirements in manufacturing and curing Blue cheese, attempts have been directed towards more economical and efficient methods of production. Quick Ripened (QR) Blue cheese loose curd offers a superior alternative to the use of Blue cheese flavor. The Blue cheese curd produced by this technique is ready for utilization in approximately two weeks compared to traditional commercial methods which require three months to one year. Although considerable progress has been achieved in modifying, improving, refining and evaluating the quick ripening process, other problems remain to be solved. The labor intensive preparation process, storage space requirement, the risk of microbial contamination prior to packaging, and control of the acceleration of Blue mold growth and metabolism have stimulated the need for a more efficient method to manufacture loose curd Blue cheese.

This project was undertaken to investigate the feasibility of:

1. quick ripening of Blue cheese curd by selecting and maintaining the optimum atmosphere over packaged curd.
2. controlling the accelerated ripening of Blue mold cheese curd by means of low temperatures and/or the use of chemical or physical methods of preservation, such as ionizing radiation or microwaving.
3. evaluating the suitability of various flexible films and/or rigid containers for controlling the composition of the headspace gas in packaged curd during quick ripening.
4. reducing handling and post-manufacture processing. Quick ripening of Blue cheese curd by controlled atmosphere packaging could result in less warehouse space needed during the manufacturing and ripening of Blue cheese curd, possible elimination or reduction in growth of both pre-package and post-package contaminant organisms and a reduction in production costs.

LITERATURE REVIEW

Cheese flavor was described by Harper (1959) as " a complex phenomenon which results from a multiplicity of chemical changes in the cheese system". The complexity of the system is evidenced by Day and Anderson (1965), where approximately 100 flavor compounds in the volatile fraction of Blue cheese were identified by gas chromatography and mass spectrometry. The major components in the aroma fraction were methyl ketones, secondary alcohols, methyl and ethyl esters of the normal aliphatic acids. Different classes of flavor components in Blue-veined cheese can originate from several sources. The most significant flavor compounds are produced by the metabolism of microorganisms, by the action of natural milk enzymes, and by the concerted action of numerous enzymes of P.roqueforti which are involved in protein and lipid metabolism. Kinsella and Hwang (1976) reviewed the biosynthesis of flavors during Blue cheese ripening. Aspects of the flavor and manufacture of Blue-veined cheese were also reviewed by Bakalor (1962).

BLUE CHEESE FLAVOR COMPONENTS AND THEIR ORIGIN

FREE FATTY ACIDS

The degradation of milk fat forms a major source of flavor compounds in Blue cheese. Currie (1914) stated that the "peppery and spicy" taste of Roquefort cheese was due to the short chain free fatty acids, caproic, caprylic, and capric and their readily hydrolyzable salts. Caproic , valeric and butyric acids in Blue cheese were reported by Thomasaw (1947). Using gas chromatography, Coffman,*et al*. (1960) analyzed dry Blue cheese and detected butyric, caproic, caprylic, capric, isovaleric and heptanoic acids. Several investigators have obtained quantitative data for free fatty acids in Blue cheese. Morris *et al*. (1955) attributed the "pungent. peppery flavor" to free acids and their oxidative products and reported finding an average of 0.77 mg butyric acid/g of Blue cheese made from pasteurized, homogenized milk. The average caproic and higher acid value (average molecular weight 228) was 24.93 mg/g of cheese. Day and Anderson (1965), utilizing gas chromatography, reported that the major fatty acids in Blue cheese were C2 - C18:3 acids. Similar concentrations of free fatty acids were reported by Blakely (1970) in QR

loose curd Blue cheese. The lipid and fatty acid content of Blue cheese was also studied by Fujishima et al. (1971). Harte and Stine (1977) reported the same free fatty acid (butyric - linolenic) patterns between QR and commercial Blue cheese. Anderson (1966) showed a difference between the fatty acid content of Roquefort and imported Blue cheese. Roquefort flavor and aroma were more characteristic of caprylic and capric acids due to the difference in composition of the fat in sheep's milk as compared to bovine milk fat.

LIPID METABOLISM (LIPOLYSIS)

Lipid hydrolysis during cheese ripening is governed by lipase activity. The lipases in cheese may originate from milk lipases,, microorganisms from the milk, and from the interior or surface of the cheese (Stadhouders and Mulder, 1957). Various authors have demonstrated the presence of more than one natural milk lipase. Two lipase systems existing in raw skim milk with pH optima of 5.4 and 6.3 have been reported by Albrecht and Jaynes (1955). Taressuk and Frankel (1957) observed two lipase systems native to Cow's milk, a plasma lipase associated with casein and a lipase absorbed irreversibly on the fat globule membrane. Chandan and Shahani (1963) isolated a low molecular weight lipase from milk clarifier slime. This lipase had the ability to

hydrolyze milk fat within a pH range of 5.0 - 10.0. Downing and Andrews (1969) reported five lipases in bovine milk. However, those lipases with a pH optimum of 7.0 and above could not continue to be active in Blue-veined cheese manufacture (Bakalor, 1962). Jensen (1964) reported that when mixed triglycerides are hydrolyzed, there is an obvious preference for the fats containing short chain acids. Milk lipase preferentially releases fatty acids from the primary positions (1,3) of a triglyceride. Since butyrate appears to be predominantly esterified at the 3-position, release of butyrate results.

In Blue cheese manufacture, spores of Penicillium roqueforti are used to inoculate the milk or the curd. Currie (1914) stated that a water soluble lipase was produced by P. roqueforti which hydrolyzes milk fat to produce the free fatty acids characteristic of the sharp flavor of Roquefort cheese. Morris and Jezeski (1953) reported two lipase system in P. roqueforti, isolated from mycelial extract, one with a pH optimum for milk hydrolysis at 6.5 - 6.8 and one with a pH optimum at 7.0 to 7.2. Niki et al. (1966), using butter fat as a medium, found that the optimum lipase activity was obtained at pH 6.0 and 7.5 for both the intra- and extra-cellular lipases. Different strains of P. roqueforti show certain differences in their lipolytic properties. Strains with moderate proteolytic

activity and strong lipolytic activity are the most suitable for Blue cheese production (Niki, et al., 1966). Three out of eight strains studied by Graham (1958) produced well-veined cheese and good flavor. Soto, et al. (1966) found that the amounts of carbonyls and fatty acids differed according to the strain of P. roqueforti used. Therefore, proper strain selection is necessary for the production of a quality Blue cheese.

LIPASES OF OTHER MICROORGANISMS

Some investigators have suggested that other microorganisms may be involved in the release of fatty acids from triglycerides during cheese ripening. Fryer and Reiter (1967) found a weak lipolytic activity in 56 strains of lactic acid bacteria. Strains of yeast were isolated during the manufacture and ripening of Blue cheese (Devoyod and Sponem, 1970). Of the ones present, Saccharomyces lactis, Candida lipolytica, Torulopsis sphasica, and Torulopsis fragillis have the most important lipolytic properties. Khan, et al. (1966) suggested that the microbial lipases released larger amounts of unsaturated fatty acids and smaller amounts of short chain fatty acids compared to milk lipase.

Factors affecting lipolysis in Blue cheese:

Temperature:

The optimum temperature of lipase activity in P. roqueforti was found to be between 30° to 35°C by Shipe (1951), and 30° - 32°C by Morris and Jezeski (1953). The variation in the optimum temperatures for the lipase activity may be due to the difference in growth condition, fungal strain and analytical method. A combination of temperatures could be used to obtain the optimum balance between the biological and chemical processes involved in Blue cheese ripening (Morris, 1969). Higher temperatures in the early stages of ripening promoted greater production of lipase while lower temperatures in the later stages discouraged mold growth and produced hydrolysis and/or Beta-oxidation of the free fatty acids. Kinsella and Hwang (1977) reported that the lipase system of P. roqueforti has shown to be active at low temperatures of cheese ripening (10°C).

Acidity:

The acidity of Blue cheese was studied by Coulter, et al. (1938b). A rapid reduction to pH 4.7 was observed during the first day of ripening. The pH increased after three months to 6.5, then decreased slowly to 5.7 at nine months. The marked rise in pH from about 2 weeks to 10 weeks was due to continuing proteolysis (Currie, 1914;

Morris, et.al , 1951). A tendency for the rate to decrease as the pH approached 6.5 was noted by Morris (1963). The lipase systems in Blue cheese were active in the pH range occurring throughout curing.

Salt concentration:

The effect of salt concentration on mold growth and lipolytic activity has been investigated. Gould (1941) demonstrated the inhibitory effect of NaCl on lipase activity in raw homogenized milk and cream. Levels of 5% to 8% NaCl were sufficient to inhibit lipolysis almost completely. Morris and Jezeski (1953) reported that 10-16% NaCl brine diminished lipase activity by 60%. Most organisms can not survive salt concentrations greater than 6%, and essentially none survive a concentration of 10% (Morris, 1969). Poznanski, et.al (1967) reported that the increase of salt content from 3.0% to 3.9% caused diminished acid production in Roquefort cheese. Godinho and Fox (1981 a) studied the effect of NaCl on the germination and growth of P. roqueforti. Germination of all strains (excluding "Dairy land" strain) isolated from several Blue cheeses was inhibited by >3% NaCl. Godinho and Fox (1981a) studied the salt diffusion rates and mold growth in ripening of Blue cheese. In the manufacture of Blue cheese, the amount and timing of salt application played an important and critical role in the development of the mold and therefore in the

flavor of the cheese (Kondrup and Hedrick, 1963; Godinho and Fox, 1981a).

Homogenization:

Homogenization of milk prior to Blue cheese manufacture is a critical process in the hydrolysis of milk fat. Homogenization increases the surface area of the milk fat, thus allowing increased lipolysis by milk and mold lipase systems and also alters the composition of the fat globule membrane. Lane and Hammer (1938) reported that homogenized milk yielded cheese which ripened faster, was softer in texture and lighter in color. Morris, et.al (1963) showed the importance of homogenization with progressive accumulation of free fatty acids during Blue cheese ripening. Dozet, et.al (1972) determined the effect of milk homogenization in manufacture of soft white cheese. The triglycerides in non-homogenized raw milk are not available to lipase due to the intact "membrane" surrounding the fat globule in multi-layers which inhibit attack and hydrolysis. Kinsella and Hwang (1976) reported that homogenization expands the total surface area of milk fat available for lipase attack.

The gas requirements of mold:

The necessity of having exchange of carbon dioxide, produced by the mold, with oxygen from the air during

ripening is an important factor involved in maintaining proper mold growth. Golding (1937) stated that the presence of CO_2 rather than the absence of O_2 is the limiting factor in the growth of P. roqueforti in the cheese. The author (1937) reported that the gas requirements of P. roqueforti are functions of both concentration and temperature and varied with different gases. Thom and Currie (1913) reported that P. roqueforti was able to grow at room temperature, with only slight restriction, in an atmosphere of 75% CO_2 and 5% O_2. Brown (1922) found that low temperatures increased the inhibitory power of CO_2 on the growth of mold, with no mold growth in pure CO_2. Durrell (1924) showed that CO_2 in amounts up to 5% stimulated mold growth (Basisporium gallarum). Karsner and Saphir (1926) found that concentrations of O_2 of 76% and more had a definite inhibitory effect on certain molds. Golding (1940 II) studied the oxygen requirements of P. roqueforti in an atmosphere diluted with nitrogen and carbon dioxide-free air. The author (1940 II) concluded that only with the greatest O_2 dilution (2.1% O_2) that a significant reduction (between 16% and 54%) of growth was recorded. No significant reduction of growth was observed in an atmosphere of 4.2% O_2 in nitrogen. There was a tendency for a limiting effect of oxygen on growth to be observed at higher temperatures. Golding (1940 III) concluded that relatively small

concentrations of CO_2 in air increased the growth of some P. roqueforti strains, while large concentrations inhibited growth. The acceleration of growth due to low CO_2 concentrations took place sooner at lower temperature, while the inhibition of growth, due to high concentrations of CO_2, is apparent at low temperatures sooner than at higher temperatures. Kinsella and Hwang (1976) summarized the optimum conditions for generation of methyl ketones from fatty acids by spores and mycelium of P. roqueforti. At concentration of more than 5% O_2 for the spores, more than 4% O_2 for the mycelium and more than 0.033 CO_2 was needed. The authors (1976) stated that carbon dioxide may actually enhance ketone formation by inhibiting the normal oxidation of acetyl-CoA via the Citric acid cycle, thereby causing a "back-up" of the fatty acid oxidation pathway.

Volatile acidity:

The presence of acids in very low concentrations has been identified in Blue cheese (Anderson, 1966). Table I lists some of the acids found in Roquefort and Gorgonzola cheese (Schormuller, 1968). Bassett and Harper (1958) identified acids such as oxaloacetic, oxalosuccinic and alpha-acetolactic in Blue cheese.

Table I

Some acids found in Roquefort and Gorgonzola cheese

Lactic	Succinic
Fumaric	Acetic
Propionic	iso-Butyric
alpha-Ketoglutaric	Oxalic
Phenyl propionic	Malic
p-Hydroxyphenyl pyruvic	Formic
p-Hydroxyphenyl propionic	n-Valeric
alpha-Ketobutyric	Pyruvic

Many microorganisms have the ability to carry out glycolysis, which resulted in the formation of lactic, acetic and propionic acids. Succinic, fumaric, glycoxalic, pyruvic and alpha-ketoglutaric acids are intermediate stages in the acetic acid cycle. Free amino acids are decomposed by trans- deamination to form alpha-hydroxybenzoic and other keto acids. Keto acids in Blue cheese can also be formed through citric acid fermentation and beta-oxidation. The aroma and taste of these acids differ, but they may contribute to the overall flavor of Blue cheese.

METHYL KETONES

Many researchers have studied the occurrence of methyl ketones in milk and dairy products. Starkle (1924) reported

the presence of 2-heptanone and 2-nonanone in Roquefort cheese. The author (1924) postulated that these methyl ketones were formed by the metabolism of fats by P. roqueforti and that they contributed to the flavor and aroma of this cheese. Patton (1950) isolated acetone, 2-pentanone, 2-heptanone, and 2-nonanone from Blue cheese. It was felt that these methyl ketones, particularly 2-heptanone had an aroma typical of Blue cheese (Hammer and Bryant, 1937). Morgan and Anderson (1956) identified odd-numbered methyl ketones, C_3 - C_{11}, in Roquefort, Gorgonzola, Danish and domestic cheese. Harper and Bassett (1959) surveyed the acidic and neutral carbonyl compounds in Blue cheese. Several even-numbered methyl ketones were isolated from Blue cheese such as 2- butanone, 2-hexanone, and 2-octanone (Nawar and Fagerson, 1962). Day and Anderson (1965) confirmed the presence of methyl ketones: C_3, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11} and C_{13} in Blue-vein cheeses by means of gas chromatography and mass spectral analysis. Schwartz and Parks (1963) quantitated the odd-numbered methyl ketones from C_3 to C_{15} in domestic Blue cheese using chromatographic methods. Schwartz, et al. (1963) made similar measurements on Roquefort cheese and 2-heptanone was the major ketone in all samples except one which contained more 2-nonanone. Quantitation of the methyl ketones in Blue-veined cheeses, by Anderson and Day (1966), indicated a large variation in

the quantity of ketones in different cheeses, with 2-heptanone being the most abundant ketone in all samples. Blakley (1970) reported a much lower concentration of methyl ketones, C_3 to C_{13}, in quick ripened (7 days) Blue cheese. The author (1970) found 2-nonanone to be the major methyl ketone in Danish and quick ripened Blue cheese. In the same study, the author found 2-undecanone to be the most abundant ketone with slightly smaller amounts of 2- nonanone present in quick ripened cheeses made from filled milk containing hydrogenated coconut oil. A much higher concentration of methyl ketones in quick ripened Blue cheese and lower content of methyl ketones in commercial Blue cheese were reported by Albert (1974). Dokhani (1978) quantitated C_3 to C_{13} methyl ketones in quick ripened and commercial Blue cheeses utilizing a high performance liquid chromatographic system. The author (1978) concluded that the predominant methyl ketones were C_7 and C_9. Starkle (1924) investigated the ability of several mold cultures, Penicillium glaucum, P. roqueforti, Aspergillus niger, and A. fumigatus, to convert free fatty acids to methyl ketones, C_7, C_9 and C_{11} from oxidative decomposition products of cocoa fat and butter and compared them with ketones obtained by hydrogen peroxide oxidation of pure fatty acids. A mechanism for the formation of these ketones was advanced by Stokoe (1928). The author (1928) suggested that Penicillium species oxidize

a chain compound primarily at the beta-carbon atom with the formation of the beta-keto acid followed by decarboxylation to the methyl ketone and carbon dioxide.

Mechanism of Methyl Ketone Formation by Fungi:

Gehrig and Knight (1958, 1961, and 1963) demonstrated that only spores of P. roqueforti were capable of producing methyl ketones from fatty acids. The capacity to form methyl ketones disappeared rapidly and progressively as the spores germinated. In contrast, Lawrence and Hawke (1968 reported the formation of methyl ketones by mycelia of P. roqueforti from low concentrations of fatty acids with less than 14 carbon atoms over a wide range of pH. Dwivedi and Kinsella (1974a and 1974b) carefully obtained mycelia cultures of P. roqueforti and demonstrated the ability of mycelia to oxidize fatty acids and produce methyl ketones. Fan, et al. (1976) showed the relative rates of methyl ketone formation from fatty acids by spores of P. roqueforti at progressive stages of germination. They concluded that mycelia were much more active under short incubation conditions; however, the resting spores showed very high activity during a long period of incubation. Lawrence (1966) reported that the addition of Casamino acid or certain amino acids (particularly L-proline or L- alanine) greatly stimulated oxidation of triglycerides by spores of P. roqueforti. The

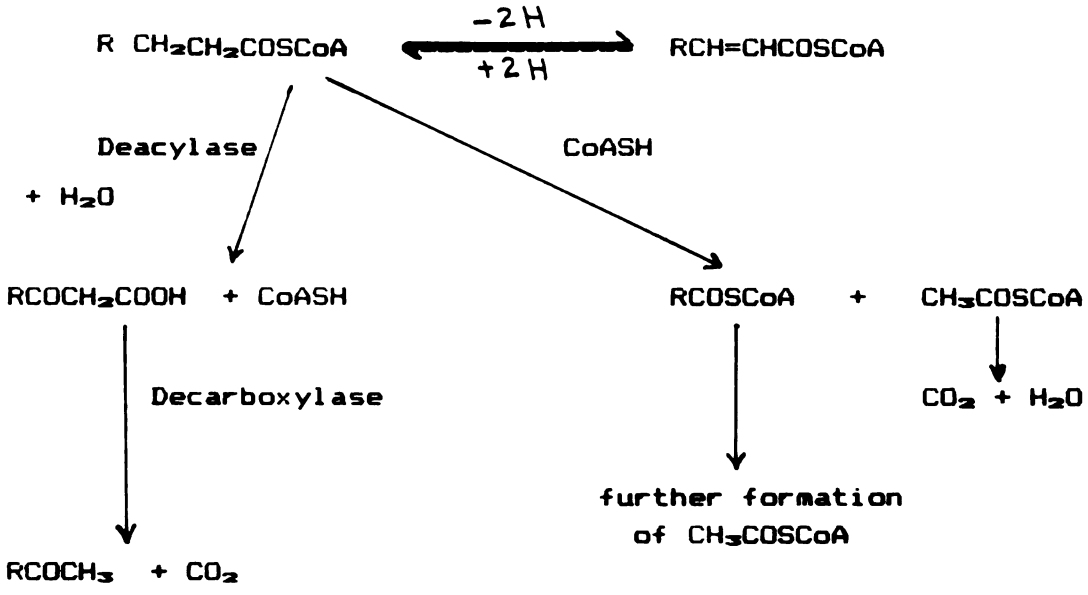
rate of oxidation was further increased by addition of certain sugars (particularly D-galactose, D-xylose, D-glucose) to the media. The increase in the rate of 2-undecanone formation by the addition of glucose to the resting spores of P. roqueforti was shown by Hwang, et al. (1976). Using 1-^{14}C labeled octanoate as substrate, Gehrig and Knight (1963) demonstrated that in small concentration (1 uM), sodium octanoate was oxidized completely by spores of P. roqueforti; however, in larger concentrations (20 uM), part of the molecule was converted to methyl ketone and part was completely oxidized. The authors (1963) stated that no ketone was produced under anaerobic conditions. Wong, et al. (1958) proposed that the methyl ketones obtained from milk fat might be derived from beta-keto acids which are natural constituents of milk fat. Hawke (1966) outlined a schematic pathway of fatty acid oxidation for methyl ketone formation similar to the classical beta-oxidation cycle of fatty acids as shown in scheme I. This mechanism provides for the formation of:

1. only methyl ketones of one less carbon atom than the fatty acids used as substrates.
2. a simultaneous process for B-oxidation of fatty acids.

Scheme I

Pathway of Fatty Acid Oxidation

and Methyl Ketone Formation



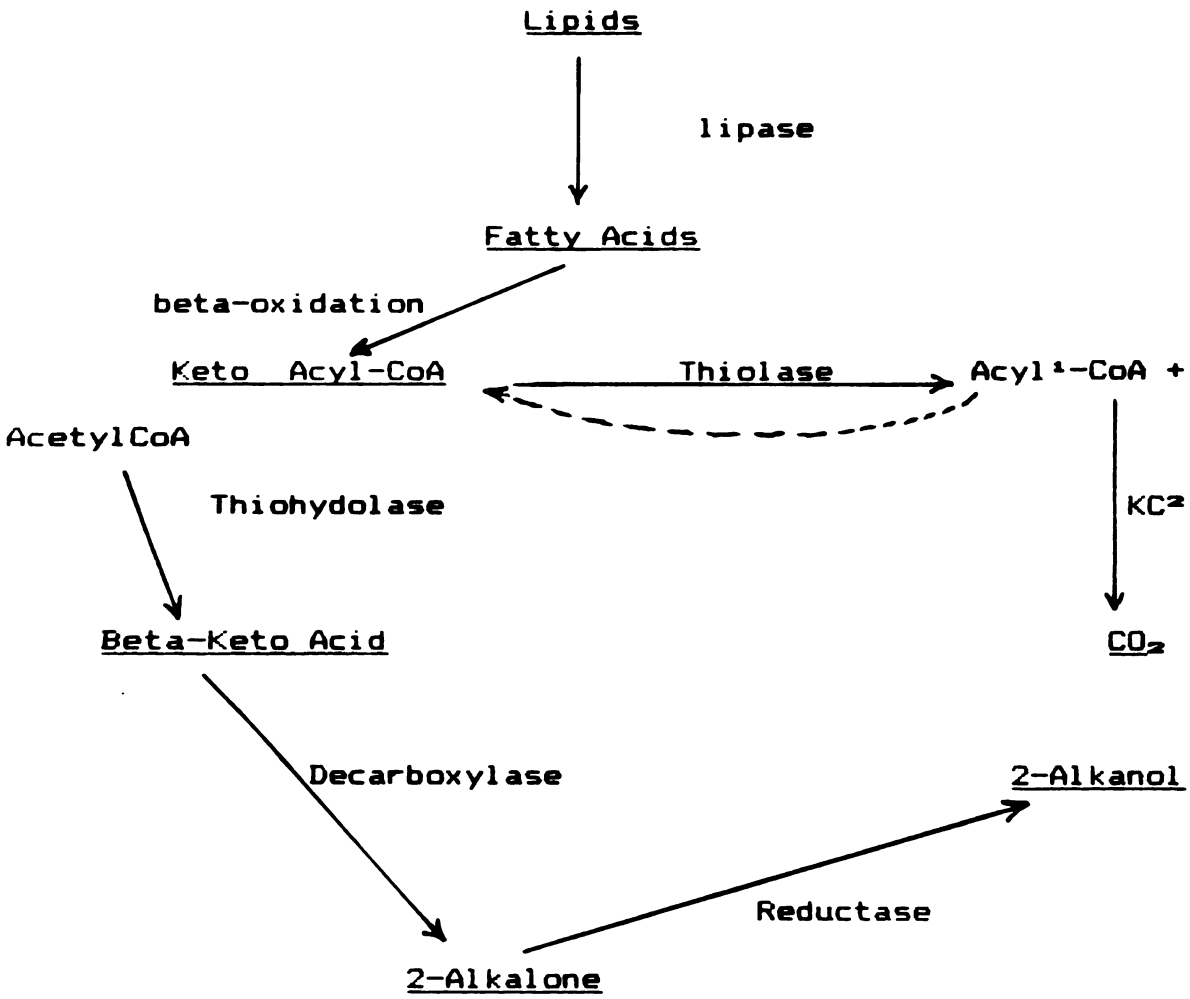
Kinsella and Hwang (1976, 1977) diagramed the metabolism of fatty acids by P. roqueforti as it occurs in Blue cheese (scheme II). These authors (1976,1977) described the following steps, which are similar to the process outlined by Hawke (1966):

1. Lipolysis of triglycerides to form free fatty acids .
2. Dehydrogenation of the beta-Hydroxy acyl-CoA to form beta-Keto acyl-CoA.
3. beta-Keto acyl-CoA is deacylated to form beta-Keto acid and CoASH by beta-Keto acyl-CoA deacylase or Thiohydrolase.

4. Rapid decarboxylation of beta-Keto acid to methyl ketones (2-Alkanones) and carbon dioxide by beta-keto acid decarboxylase.

5. The 2-Alkanones produced are easily reduced to the corresponding 2-Alkanols (secondary alcohols) by a reductase.

Scheme II



¹cyl group may contain from 4 to 16 carbon atoms.
²C represents the Krebs or Citric acid cycle.

The Thiolase is possibly inhibited by Acyl-CoA (denoted by the broken line in the scheme), thereby facilitating the deacylation-decarboxylation step (Kinsella and Hwang,1977). The effect of environmental parameters on the rate of beta-oxidation was demonstrated by Lawrence (1966). The author (1966) observed that the pH optimum for methyl ketone formation varied with the concentration and type of fatty acids. The maximum yield of methyl ketone obtained was 25% from trioctanion and trihexanion at pH 6.0. Hwang, et al. (1976) reported an optimum pH, 6.5 - 7.0, for 2-undecanone formation from beta-ketolaurate. Lawrence (1966) reported an optimum temperature of 27°C for the maximum rate of methyl ketone formation. Gehrig and Knight (1963) suggested an optimum temperature of 25°C for methyl ketone production.

Other Flavor Compounds

In addition to methyl ketones and free fatty acids, secondary and tertiary products have been found in Blue cheese. Secondary alcohols were identified by Stokoe (1928) as a result of the action of P. roqueforti on coconut oil. The author (1928) postulated that these alcohols were intermediate products in the oxidation of fatty acids to methyl ketone. He also considered the possibility that they were formed by the reduction of ketones. Jackson and Hussong (1958) isolated 2-pentanol, 2-heptanol, 2-octanol

and 2-nonanol from Blue cheese. Anderson and Day (1965) identified 2-propanol, 2-pentanol,2-heptanol, 2-octanol and 2-nonanol as components of Blue cheese aroma. Nawar and Fagerson (1962) reported the presence of 2-pentanol and 2-heptanol in Roquefort cheese. Anderson and Day (1966) determined the concentration of secondary alcohols in Blue cheese. The alcohols were found in much lower concentrations than the methyl ketones. Kinsella and Hwang (1977) suggested that a musty flavor in Blue cheese may result when 2-alkanols are present in large concentrations. Acetaldehyde, 2-methylpropanal, 3-methylbutanal and furfural were identified by Anderson (1966). The presence of these aldehydes may be due to the action of enzymes involved in the oxidative deamination of amino acids (Kinsella and Hwang, 1977; Anderson, 1966).

Ethanol, acetyl methyl carbinol, and diacetyl have been identified in Blue cheese by Bassett and Harper (1958). Methanol, ethanol, n-pentanol, 2- and 3-methylbutanol, and 2-phenylethanol were identified in Blue cheese by Day and Anderson (1965).

The importance of methyl and ethyl esters in the overall flavor profile of Blue cheese was reported by Anderson and Day (1966). Different analogs of methyl, ethyl, and isopropyl esters were identified by Anderson (1966). Jolly and Kosikowski (1975) identified and

uantitated five different lactones in Blue cheese ontaining added microbial lipase.

ROTEIN METABOLISM (PROTEOLYSIS)

Proteolysis (degradation of protein) is important in cheese ripening for the development of proper texture, for background flavor (peptides and amino acids), for provision of amino acids which act as precursors of flavors (aldehydes, alcohols and esters) and as germination stimulants (Kinsella and Hwang, 1977). The extent of protein degradation varies with the variety of cheese. Primary protein hydrolysis to amino acids occurs to some extent in all cheese varieties. In Blue cheese, proteolysis varies with the strain of P. roqueforti, the temperature of ripening and the pH. Porks et al. (1959) reported that the strains of P roqueforti differed in their lipolytic and proteolytic activities. Niki et al. (1966) observed that strains of P. roqueforti possessing low lipolytic activity exhibited high proteolytic activity and vice versa. The authors (1966) suggested that strains with low proteolytic and high lipolytic activity are most suitable for Blue cheese manufacture. Czulak (1959) attributed a marked bitterness in cheese to the accumulation of certain peptides and peptones. Niki et al. (1966) reported that strains which hydrolyzed over 50% of the protein produced soft

cheeses with an unpleasant bitter taste. Niki et al. (1966) detected the presence of an intra and extracellular protease with an optimum pH of 5.5. The extra-cellular protease contributes to casein degradation during the early stages of ripening, whereas the intra-cellular protease contributes to proteolysis throughout the entire ripening period. Modler et al. (1974 b) isolated and examined a pure extra-cellular protease. Modler et al. (1974 a) reported that pH optima of extra-cellular proteases were different on different substrates. The authors (1974 a and 1974 b) demonstrated a wide range of pH's (3.0 - 6.0) for maximum stability of the extra-cellular protease and showed the optimum temperature to be 45° to 46°C. Using a protease isolated from Penicillium, Gripon and Bergere (1972) reported two pH optima, 3 and 6, for casein hydrolysis. The optimum temperature ranged between 35° and 43°C. The activity of the protease fell off below 25°C. Therefore, ripening temperatures of 10-15°C minimize excessive amino acid and peptide accumulation in Blue cheese. Dominique and Gripon (1981) demonstrated the action of P. roqueforti aspartyl proteinase on purified caseins and identified the main degradation products obtained from beta-casein. The authors (1981) demonstrated also the activity in cheese of the two endopeptidases (aspartyl and metalloproteinase) using commercial Blue cheeses.

Sodium chloride added to cheese also affects proteolysis. Gripon and Hermier (1974) demonstrated the inhibition of extra-cellular protease by adding sodium chloride to the medium. Godinho and Fox (1981 b) studied the diffusion rates of sodium chloride into Blue cheese curd and the influence of salt concentration on the growth of P. roqueforti in cheese. The authors (1981 b) reported that the salt concentration in the outer layer of the cheese decreased, presumably as a result of continued inward diffusion of salt. The mold development was affected by the rate of diffusion of salt. A slower rate of diffusion in such cheese permits spore germination.

The breakdown of protein is progressive during cheese ripening. Jolly and Kosikowski (1975) reported that soluble nitrogenous compounds comprised about 25% of the total nitrogen in ripened blue cheese and 34% of cheese made with added protease. Ismail and Hansen (1972) reported that the free amino acid levels in samples of Danish blue cheese ranged from 5 to 10% of the total nitrogen. Kuehler (1974) determined the concentration of free amino acids in quick-ripened Blue cheese samples. The amino acid content increased to 28% of the total protein after ripening 39 days compared with 16% after 11 days ripening. Takafuji et al. (1982 b,c) studied the action of intracellular neutral proteinases from P. camemberti on casein fractions. The

authors (1982 b,c) concluded that initially alpha$_s$-casein is broken down by Chymosin, and after 20 days beta-casein is broken down by fungal proteinase, releasing high molecular weight peptones. The hydrolysis of beta-casein by the metalloproteinase and aspartyl proteinase from P. roqueforti was observed when mold growth was apparent in Bleu d' Auvergne cheese (Trieu-Cuot and Gripon, 1983). Hewedi and Fox (1984) reported that proteolysis in Blue cheese was rapid between 10 and 16 weeks presumably due to the proteinases/peptidases excreted by P. roqueforti. Approximately 67% of the total nitrogen in commercial mature Blue cheese was water soluble.

ACCELERATION OF RIPENING AND/OR IMPROVING THE FLAVOR OF CHEESE

Various methods have been described for shortening the period during which desired texture, color and flavor are obtained in Blue cheese. Lane and Hammer (1937) investigated the effect of homogenization of cheese milk on subsequent flavor development. The authors (1937) observed a more uniform flavor development in the cheese made with homogenized milk compared with unhomogenized raw milk. Cheese made from pasteurized-homogenized milk showed a rapid development of volatile acids and a more typical flavor than

cheese made from unhomogenized raw milk. Morris et al. (1955) reported a 4 - 5 month ripening period for Blue cheese made with raw or pasteurized-homogenized milk compared to a 9 - 11 month ripening period with unhomogenized raw milk. Peters and Nelson (1961 a) demonstrated that ripened Blue cheese made from homogenized milk, both raw and pasteurized, was higher in quality than ripened cheese made from raw or pasteurized nonhomogenized milk. The same authors (1961 b) studied the effect of different ripening temperatures on the quality of Blue cheese. A procedure was developed by Kondrup and Hedrick (1963) and patented by Hedrick et al. (1968) to shorten the ripening period of Blue cheese from the conventional 90 days to 10 days. The mold was inoculated into milk after setting. Loose curd rather than the normal hooped wheels was ripened at 62°F and 95% relative humidity. Salting was performed in three stages. The curd was agitated daily until a desirable texture, color and flavor was obtained. The cured product was packaged in polyethylene bags with a capacity of 25 pounds and stored at 45°F. Blakely (1970) prepared a quick ripened filled Blue cheese. The procedure used was that of Kondrup et al. (1963) except that coconut oil was used to replace milk fat. A high percentage of particular fatty acids and methyl ketones was obtained. The flavor was described as not completely typical of Blue

cheese. A modified procedure of Hedrick et al. (1968) was used by Kuehler (1974), Harte (1974), Albert (1974) and Harte and Stine (1977) to produce quick ripened Blue cheese with more desirable quality. The authors concluded that homogenization and pasteurization of milk, cooking the cut cheese curd, salting times and temperature of curing were critical for development of a clean, full flavor and control of contamination. Various methods can be used to accelerate cheese ripening. Sood and Kosikowski (1979) reported that the addition of microbial enzymes, fungal protease and fungal lipase to Cheddar cheese curd accelerated the ripening process.

Law (1978, 1980) reviewed the possibilities of accelerating cheddar cheese ripening by the use of non-conventional starters and enzymes. The author (1978, 1980) described several methods such as addition of enzymes (microbial proteinases and lipases), increased microbial populations by addition of modified lactic acid bacteria to cheese milk or addition of bacterial concentrates to the curd, and the use of mutant organisms. One notable alternative to increasing enzyme concentrations is to increase the activities of existing enzymes by changing the temperature and moisture levels artificially in slurry systems.

The use of lactose to stimulate the growth of normal

culture, and the high glucose availability probably encourages the growth of other flavor-promoting microorganisms (e.g. molds in Blue cheese). This principle was applied to shorten the ripening time and speed up the flavor development of several cheese varieties including Cheddar, Mozzarella, Camembert and Blue (Anon, 1977). Law and Wigmore (1982) reported that development of Cheddar cheese flavor was accelerated by the addition of bacterial neutral proteinase to the curd. The authors (1982) demonstrated that the addition of fungal acid proteinase resulted in a bitter defect even at low concentrations. Davide and Foley (1981) and El-Soda et al. (1981) reported that the use of disintegrated frozen bacterial cell concentrate (DFCC) and cell free extract (CFE) or cell debris (CD) to supplement the normal starter in Cheddar cheese accelerated proteolysis with no effect on glycolysis or lipolysis. El-Safty and Ismail (1982) demonstrated that addition of free fatty acids to Domiati cheese milk improved cheese flavor and shortened the ripening period. Kalinowski et al. (1982) reported that complex enzyme preparations consisting of P. candidum and S. lactis autolysate were used to accelerate ripening of Dutch, Tilsit and lowicki-type cheeses. Grieve and Dulley (1983) used Streptococcus lactis Lac^- mutants to accelerate Cheddar cheese ripening. Furtado (1983) reported that Camembert-type cheese was

ripened in a loose curd at 13°C and 95% RH for 21 days. The author (1983) reported that the amounts of volatile fatty acids and soluble protein in the experimental cheese were higher than in commercial cheeses. The addition of trace elements has accelerated the ripening rate of Ras cheese (Maghdoub et al., 1985). Farahat et al. (1985) reported that the ripening period of Ras cheese was reduced to 2 months, compared with 4 months for the control by pre-treatment of cheese milk with beta-D-galactosidase (lactozym). Upadhyay et al. (1985) reported that higher ripening temperatures can accelerate Cheddar cheese ripening. The flavor and body/texture scores and total volatile fatty acid levels of cheeses ripened at 12 - 13°C for 4 months were similar to those of cheeses ripened at 5-6°C for 6 months. Aston et al. (1985) reported that the maximum temperature at which Cheddar cheese could be stored for 32 weeks without reduction in quality was 15°C. Bartels et al. (1987) reported that the addition of heat-shocked thermophilic lactobacilli and streptococci to the regular lactic starter accelerated flavor development and proteolysis in Gouda cheese.

The acceleration of cheese ripening represents a worthwhile saving in the total cost of cheese manufacture and storage provided that flavor development can be

accelerated without impairment of flavor balance (Jameson, 1983).

Control of accelerated ripening

It is critical to control the accelerated cheese ripening since a cheese which matures quickly is also likely to become over-ripe quickly and methods to arrest ripening are not readily available (Law, 1980). In quick ripened Blue cheese, excessive mold growth frequently occurs and this results in the development of a musty, moldy flavor. The possibility of unwanted microbial contaminants may also occur. Jameson (1983) stated that maturation of cheese may be retarded by storage at low temperature, but freezing usually causes breakdown of the cheese structure, resulting in a short, crumbly or mealy body. Stehle (1984) reviewed the possibilities of prolonging storage life of Camembert cheese by heat treatment involving surface pasteurization, microwave heating, autoclaving, irradiation, freezing, and high-frequency heat treatment. Herrmann and Rudolf (1980 a, b) demonstrated in laboratory and full-scale experiments that refrigerated storage of Camembert-type cheese (at 0°-4°C and 65 - 85% RH, in laminated aluminum foil packaging) from the 10th to 39th day after manufacture, considerably increased storage life. Slanover (1981) reported that cheeses cooled immediately after salting were

unsatisfactory. The author (1981) suggested an initial ripening period before refrigerated storage. Jarmul et al. (1985) reported that Camembert cheeses which were frozen and stored at -27°C at 75% RH for 2 - 3 months and then ripened for 1 -3 weeks, after thawing, yielded cheese of satisfactory body and flavor. Tochman et al. (1985) studied the effect of microwave heating "in-package" on the quality of Cottage cheese. The authors (1985) concluded that optimum quality was observed when the packaged cheese was heated to 48.8°C using a 0.5 KW power source.

Kreal et al. reported that irradiation of Bryndza cheese at more than 100 krad resulted in substantial reductions in the counts of all microorganisms, but caused flavor defects (burnt, foreign and unclean). Yuceer and Gunduz (1980) reported that preservation by irradiation combined with refrigeration increased the shelf-life of Kashar cheese about five fold. Boyar and Gnanayutham (1981) reported that surface irradiation of cheese using an electron beam facility lead to a delay in mold growth, but the irradiated cheese developed off-flavor at the surface. Ibrahim et al. (1987) reported that microbial counts decreased when Ras cheese was exposed to gamma-irradiation of 0.25 M rad at 0.10 M rad/hr. The optimum day to irradiate Ras cheese was day 60 of ripening.

Modified- and Controlled Atmosphere Packaging

The terms "modified atmosphere" (MA) and "controlled atmosphere" (CA) mean that the atmospheric composition surrounding a perishable product is different from that of normal air (Brecht, 1980). (MA) differs from (CA) only in how precise gas partial pressures are controlled. The initial gas concentrations in (MA) systems are established but not readjusted during storage, while in (CA) systems the selected concentrations of gases are maintained throughout storage (Wolfe, 1980, Ogrydziak and Brown, 1982). The manipulation of the environment can take place in an (MA) system, where an actively respiring and metabolizing product reduces the oxygen and increases the carbon dioxide in the ambient air within a chamber in which various barriers and restrictions to gas exchange exist (Smock, 1979). Three barriers exist: the product itself, the package and the storage room. Lee (1987) stated that fresh produce respires at a given rate under constant temperature. If the package allows the influx of oxygen and efflux of carbon dioxide at the same rate as that of the fresh produce, a constant concentration of oxygen and carbon dioxide can be maintained inside the package. The author (1987) reported that the influx of oxygen and efflux of carbon dioxide are determined by the permeability of the package, thickness and surface area of the film, and the partial pressure gradients

inside and outside the package. The respiration rates vary with type of product and storage conditions. A dynamic equilibrium exists between the endogenous gases produced by the product and the exogenous gases surrounding the product (Burton, 1978). The balance between exogenous and endogenous gases influences the rate of diffusion into and out of the product. Dynamic changes due to gas permeation, absorption, and/or bio-chemical conversion via respiratory activity must be also considered (Wolfe, 1980).

Effect of modified- and controlled atmosphere

A number of studies have been conducted to determine the effects of modified- and controlled atmosphere on metabolism and microorganisms growth.

Smock (1979) and Burton (1978) reviewed the influence of (CA) on respiration rate and other basic catabolic processes of stored fruits and vegetables. They concluded that the effects of (CA) systems on respiration were dependent on the plant material and on the concentration gradient that develops between the centers of metabolic action and the outer integument of the plant material. Oxygen levels ranging from 3 to 21% have been shown to influence the Krebs cycle, whereas levels below 3% also inhibit the glycolytic system (Brecht, 1980). An oxygen level of 2.1% was shown to reduce the growth of P.

roqueforti significantly (Golding, 1940 II). The respiration rates of a number of different organisms as a function of the carbon dioxide tension in the storage atmosphere have been studied (Gill and Tan, 1980). The organisms which were studied included common spoilage organisms such as Pseudomonas, Acinetobacter, Enterobacter and Microbacterium thermosphactum. The authors (1980) concluded that the level of carbon dioxide that gave maximum inhibition for the common spoilage organisms was approximately 200 mm-Hg, equivalent to 26% CO_2 in air at 30°C. Silliker and Wolfe (1980) reported that lactic acid bacteria, such as streptococci and lactobacilli, are less affected by elevated levels of CO_2 and comprise the predominating flora on meats stored in modified atmospheres. Goldings (1940 III) concluded that carbon dioxide concentrations up to 10% increased the growth of strains of P. roqueforti in seven days at various temperatures. The effect of temperature on growth of microorganisms in relation to CO_2 concentrations has been studied. The acceleration of growth of P. roqueforti due to low concentrations of CO_2 and the inhibitory effect due to high concentrations of CO_2 occur sooner at lower temperatures of growth than at higher temperatures (Golding, 1940 III). The rate of P. fluorescens growth on complex medium in 20% CO_2 in air was inhibited by 80% at 5°C compared to 20% at 30°C

(Gill and Tan, 1979). The increased inhibitory efficiency of carbon dioxide at lower temperatures is related to the dissolution of CO_2 into the medium (Finne, 1982). Since the solubility of gases is much higher at low temperature, the CO_2 concentration in the medium will increase as the temperature is lowered. Harned and Davies (1943) reported that the solubility of CO_2 decreases as the salt concentration of a solution increases. The authors (1943) reported that the solubility of CO_2 at 0°C in 0.2, 0.5 and 1.0 M sodium chloride solutions is 94.2, 86.7 and 76.4%, respectively, of the solubility in water. The CO_2 molecule is more soluble in water than are most other gases. More than 99% of CO_2 exists as the dissolved gas in water (Knoche, 1980). Less than 1% exists as H_2CO_3, which partly dissociates to give H^+, HCO_3^- , and $CO_3^=$.

Advantages of modified and controlled atmosphere

Various beneficial effects of modified and controlled atmosphere have been reported. Examples of such effects are: retarding senescence (ripening and aging), reducing product sensitivity to ethylene and reduction in the incidence and severity of decay of fruits and vegetables (Brecht, 1980; Smock, 1979), extending the shelf life of poultry,meat and seafoods (Wolfe, 1980; Ogrydziak and Brown, 1982), and inhibiting the growth of Gram-negative bacteria

and other related psychrotrophs which produce off odors and flavors (Silliker and Wolfe, 1980). Extension of product shelf life by Controlled Atmosphere Packaging (CAP) has also great economical advantage to retailers (Hansen and Duckworth, 1982). Controlled Atmosphere Packaging allows a full range of products to be kept on display for a long time which allows products to be packed in a central location and in turn releases the floor space needed for preparation and packaging and increases the availability of the sales area. An advantage which might be derived from modified or controlled atmosphere package is the enhancement of product properties. This may be accomplished by selecting or maintaining the optimum atmosphere over the packaged product. Once the optimum gas mixture required by the product is established, a package material with optimum barrier properties (gases and water vapor transmission) for the product can be selected. In the process of selecting the optimum package material, parameters such as the respiration rate and/or the respiration quotient of the product as defined by the ratio of carbon dioxide evolved to oxygen consumed (Karel, 1974), composition of the product, and storage conditions (temperature, relative humidity, light,etc.) become very critical. These parameters affect both the selection of package material and the metabolic activity of the product (Karel, 1974).

Cheese Packaging

Three methods currently predominate the film packaging of cheese at the retail level: tight wrapping, vacuum-gas flushing, and vacuum packaging. Pearson and Scott (1978) provided an excellent comparative discussion of the three methods. Tight wrapping involves either machine or hand wrapping of plastic films or foils around cheese portions followed by heat sealing (Pearson and Scott, 1978). Tight wrapping utilizes less expensive materials and is applicable to many different cheese shapes, sizes and textures. Packaged cheeses will also stack more easily and thus optimize available space. The disadvantages associated with tight wrapping are that it is time consuming and the materials utilized are more easily punctured than more complex laminated structures (Pearson and Scott, 1978). Vacuum packaging involves the removal of air from the surface of perishable food stuffs, such as bacon, cooked meats, continental sausages, cheeses,etc. and the pack sealed under a vacuum (Ross, 1982). This method is widely recognized in the cheese industry and is often employed at one stage or another in the handling and storage process (Anonymous, 1977).

A variation of vacuum packaging is called gas flushing. In this process, air is removed from the package and an inert gas is then introduced. A single gas such as nitrogen

may be employed, or a mixture of gases such as carbon dioxide and nitrogen, or for some applications, oxygen, to preserve or enhance the properties of the product (Anonymous, 1977; Ross, 1982). This process is generally used for small portion size packs of the product at point of sale, although on occasion, three hundred pound drums or barrels of product are gas packed. A gas flushing operation might be a possibility in facilitating cheese ripening provided that the gas mixture required for optimum ripening is established and the package material chosen meets these requirements.

Packaging Material Requirements

Shaw (1977) reported that packaging films should be suitable for printing, contribute no off flavors or odors to the cheese and of course must not contain toxic substances capable of migration into the food. The author (1977) concluded that toxicological safety data must be provided by the manufacturer to the Food and Drug Administration (FDA) before approval of the materials for use in food packaging. Ionson (1979) stated that an extremely important requirement of packaging films is that they adequately control the headspace environment of the packaged cheese. Seal integrity, barrier properties of the film, extent of air entrapment and gas production by the cheese will influence

the extent of mold development and can be controlled by selection of suitable films. If the atmosphere within a package is oxygen deficient and/or carbon dioxide rich, the metabolism of Blue cheese will be suppressed. Metabolism can be depressed by decreasing oxygen concentration, decreasing temperature, increasing carbon dioxide concentration, or any combination of the three (Kidd and West, 1974).

Gas flush systems require complex laminates. The components of laminated films are chosen so that each component contributes a property or properties desirable to the total system (Ionson, 1979). For example, PVC/nylon or PE/nylon are often used as components of laminates and provide excellent barrier and flexural properties. When selecting a film, it is vital to know the water vapor and gas permeability of the film.

Factors affecting permeability

Film, gas and water vapor permeabilities are affected by a number of physical factors. Some of these factors include the area of the film, the time of exposure and the partial pressure exerted on the film. Other factors such as temperature, relative humidity and film thickness affect permeability in a less direct manner. Karel (1974) reported that storage at high relative humidity or direct contact between hydrophilic material and cheese can

adversely affect the water vapor and gas barriers. Decreasing temperature will improve the barrier characteristics of films, the extent of change being dependent upon the particular material (Karel, 1974; Hanlon, 1983). Polyethylene is more strongly hydrophobic than the other organic films so water vapor penetrates it to a limited degree. This particular property along with its high rate of gas permeability are of considerable value in the packaging of items which require a low moisture loss, while maintaining the ability to transmit significant amounts of oxygen or carbon dioxide.

ANALYTICAL PROCEDURES

PH

The pH measurements were determined using an Orion digital pH/mv meter equipped with a combination electrode (Standard Methods for Examination of Dairy Products, 1985). Measurements were taken initially and continued on a daily basis. The pH was read to the nearest one-hundredth of a pH unit on the meter scale.

Moisture Content

The moisture content of the cheese curd was determined by the vacuum oven method (AOAC, 1085). Determinations were made in triplicate and performed initially and on a weekly basis.

Gas Headspace Analysis

Package headspace analysis was accomplished using an oxygen analyzer (Model S-3A/11, Applied Electrochemistry) coupled with a Carbon Dioxide analyzer (Model CD-3A, Applied Electrochemistry). Dow Corning silicon rubber was deposited on each pouch to serve as a septum. A syringe needle was inserted through the pouch material to withdraw a 1cc. of headspace gas and then injected into the analyzer.

Samples were taken initially in duplicate, then daily every 6 - 12 hours. Seven pouches were used for each temperature and determinations were made in duplicate.

Nitrogen (reduced) determination

Total and soluble nitrogen were determined using a micro-Kjeldahl unit consisting of a Buchi 342 control unit, Metrohm 655 Dosimat, Metrohm 614 impulsomat and Metrohm 632 pH-meter (Brinkmann). Determinations were made in duplicate and performed initially and on a daily basis.

Sensory evaluation

An experienced panel of cheese judges scored and rated the cheese curd. Flavor, color (mold appearance) and texture were evaluated. A crumbled, commercial Blue cheese, aged at least 6 months, was used as the control. It was felt that flavor was the most important criterion because QR Blue cheese curd is ideal for direct incorporation into Salad dressings, cheese dips and similar products. Color was rated second in importance and texture last. On this basis, flavor was assigned a score of 20 points, color 15 points and texture 10 points. A cheese with a score of approximately 30 out of the total of 45 was considered acceptable. Ideal mold growth is referred to in this study as a well pronounced, even distribution of mold, and blue

coloring which appears on the cheese surface and/or throughout cheese curd.

High Performance Liquid Chromatographic (HPLC) Analysis of Methyl Ketones

Purification of solvents

Hexane: Carbonyl-free hexane was prepared from redistilled hexane by the methods of Hornstein and Crow (1962), and Schwartz and Parks (1961). A column was prepared using 45 ml of concentrated sulfuric acid blended with 75 g of Celite 545 (John Manville). A small amount of anhydrous sodium sulfate was placed at each end of the column to serve as a desiccant. The eluate from this column was passed over a 2,4-DNPH-reaction column (Shwartz and Parks, 1961). Five-tenths of a gram of recrystallized 2,4-Dinitrophenylhydrazine (2,4-DNPH) was dissolved in 6.0 ml of 85% phosphoric acid by grinding. Deionized distilled water (4 ml) was added and the precipitated DNPH was redissolved by grinding. Ten grams of Celite 545 was ground with the solution until a homogeneous bright yellow blend was obtained. The mixture was packed in a chromatographic column containing 25 ml carbonyl-free hexane. The column impurities were flushed away with 50 ml carbonyl-free benzene. The eluate from the reaction column was mixed with

Sea Sorb 43 (Adsorptive magnesia, Iodine No. 80, Johns Manville) as suggested by Blakely (1970) and filtered to remove the carbonyl derivatives from hexane.

Benzene:

Carbonyl-free benzene was prepared by refluxing reagent grade benzene (Fisher Scientific Co.) with 1 g of 2,4-DNPH per 500 ml for 2 hrs. and then redistilled.

Chloroform:

Analytical grade chloroform (Fisher Scientific Co.) was refluxed over potassium hydroxide for 30 min. and redistilled.

Nitromethane:

Analytical grade nitromethane (Aldrich Chemical Co.) was redistilled over boric acid and stored.

Acetonitrile:

High purity acetonitrile (b.p. 81 - 82$_{\circ}$ C) was obtained from Burdick and Jackson Lab, Inc.

Ethanol:

Carbonyl-free ethanol was prepared by distilling 95% ethanol (Fisher Scientific Co.) over 2,4-DNPH, using 1 g 2,4-DNPH per 100 ml ethanol.

Butanol,1-:

Distilled in glass Butanol-1 was obtained from Fisher Scientific Co.

Ethylene Chloride:

Ethylene chloride (Mallinckrodt,Inc.) was redistilled over anhydrous potassium carbonate.

All the purified solvents were stored in brown bottles.

Preparation of 2,4-DNPH Derivatives of Standard Methyl Ketones:

Standard methyl ketones, 99% purity, were obtained from Aldrich Chemical Co., Inc. and were used to form hydrazone derivatives according to Shriner et al. (1964). The recrystallized 2,4-DNP-hydrazones were filtered, dried at room temperature and then stored in the freezer.

Isolation of Mono-carbonyls from Cheese samples:

Extraction of Fat:

Ten grams of a representative cheese sample was mixed with 15 grams Celite 545, dried for 24 hrs. at 150° C, and ground to a homogeneous blend. The mixture was packed into chromatographic column. The fat was extracted with 200 ml carbonyl-free hexane. A similar column was packed and extracted with 200 ml carbonyl-free hexane into pre-weighed soxhlet flasks to determine the fat recovery.

Formation of 2,4-DNP-hydrazone derivatives:

The hexane extract of the cheese sample was passed through a 2,4-DNPH reaction column to convert the carbonyl compounds in the fat extract to their corresponding 2,4-DNP-hydrazones (Schwartz and Parks, 1961). The column was washed with 200 - 300 ml carbonyl-free hexane and the solvent was removed using a rotary evaporator (Precision Scientific, Inc.).

Removal of fat from 2,4-DNP-hydrazones:

The fat containing the hydrazones was dissolved in 5-10 ml carbonyl-free hexane and the 2,4-DNP-hydrazones were isolated from the fat as described by Schwartz et al. (1963) with slight modification by Dokhani (1978). The fat was washed off the column by subsequent addition of 200 ml hexane, 100 ml hexane: benzene (1:1 v/v) and 200 ml benzene. The hydrazones, which were adsorbed on the column as a deep brown band, were eluted by adding 250 ml chloroform- nitromethane (3:1 v/v). The eluate was evaporated using a rotary evaporator.

Fractionation of 2,4-DNP-hydrazones on Weak Alumina:

The mono-carbonyl derivatives were separated from Keto-glycerides as described by Schwartz et al. (1963) on a weak alumina column (Schwartz and Parks, 1961). The DNP-

hydrazones were dissolved in 5 ml hexane and applied to a weak alumina column. The mono-carbonyl derivatives were eluted by adding 50 ml hexane-benzene (1:1 v/v). The eluate was evaporated using a rotary evaporated.

Determination of Methyl Ketones by High Performance Liquid Chromatography (HPLC):

A high performance liquid chromatographic system was used to isolate and quantitate the methyl ketones present in the mono-carbonyl fraction of the cheese samples (Dokhani, 1978).

Chromatography Equipment and Conditions:

The system consisted of a Waters Associates Model 730 Data Module, M-45 Solvent Delivery System, U6K septumless injector and a Model 441 Absorbance Detector (Pharmacia Fine Chemicals). A Bransonic 221 sonicator (Branson Cleaning Equipment Co., Shelton, CT.) was used to degas and uniformly mix the mobil phase. A reversed phase u Bondapak C18 stainless steel column (3.9 x 300 mm; Waters Associates) was employed to separate components for analysis. A Hamilton 100 ul syringe was used to inject 50 ul sample volumes. The following chromatographic conditions were used:

Temperature : ambient

Detector : U.V. monitor; 254 nm

Mobile phase : acetonitrile : water (80 : 20 v/v)

Flow rate : 1.5 ml/min

Chart speed : 0.65 cm/min

Recovery of methyl ketones:

The percent recovery of individual methyl ketones was determined in order to confirm the reliability of the extraction and chromatographic procedure. A mixture of accurately weighed standard methyl ketones: C3, C5, C7, C9, C11 and C13 (99.5% purity) were spiked into a 250 g cheese sample and subjected to the extraction and chromatographic procedure, described in the experimental section. An identical cheese sample was subjected to the same preparation and aliquots of the resulting samples were injected into the HPLC. The values were compared with the untreated C3 - C13 standard methyl ketones. The percent recovery of each standard ketone, as determined by triplicate analyses, was used to calculate ketone concentrations in the cheese samples. Peak area was used for quantitation of methyl ketones.

Gas requirements:

The respiration rate was determined experimentally as suggested by Lee (1987). The cheese was kept in airtight Mason jars (500 cc) with two ports. The air was fed into

the in-port and allowed to flow over the cheese curd and exit from the out-port. The oxygen consumption and carbon dioxide production were monitored over time. Samples were taken initially in duplicate and then daily every 6 - 12 hours. Seven Mason jars were used for each temperature and determinations were made in duplicate.

Water Vapor Transmission of LDPE and Laminate (Nylon/PE):

The water vapor transmission of the test packages was determined as described in ASTM E-96 (1980). A test specimen was placed over the mouth of standard aluminum dishes which contained desiccant and sealed with molten wax. The assembly was placed in an atmosphere of constant temperature (5°, 10°, and 15°C) and humidity (95%). The weight loss or gain of the assembly was used to calculate the rate of water vapor transmission through the materials.

Oxygen Permeation of LDPE and Laminate (Nylon/PE):

The oxygen permeation rate was determined according to ASTM D-3585 (1981) applied to plastic film and sheet using a coulometric sensor. A Mocon Oxtran 100 (Modern Controls, Inc., Minneapolis,MN.) was used to measure the oxygen permeation rate. A standard polyester film was used to calibrate a chart scale prior to testing the film samples (LDPE and Laminate).

Gamma irradiation:

The effect of gamma irradiation dose and time on Blue cheese curd was investigated using a variable flux gamma irradiator (Department of Chemistry, Michigan State University) with a Co^{60} source. Cheese samples were placed in LDPE pouches and subjected to 0.5, 1.0, 2.0 and 2.5 KGy. Cooling was accomplished by flowing compressed air into the sample cavity to maintain temperature below 27°C. The samples were stored for one week at 5°C and then analyzed for methyl ketones and sensory properties.

Microwave heat treatment:

Blue cheese curd was packed in LDPE pouches and subjected to heat treatments using a 2.8 Kw microwave source (Kenmore, model #721). Two weeks after processing and packaging, the cheese was heated "in-package" to various temperatures. The physical appearance and sensory properties were evaluated.

Procedure used for manufacturing Quick Ripened (QR) Blue Cheese

Blue cheese curd was produced according to the method of Kondrup and Hedrick (1963) and Hedrick *et al*. (1968). The modification of Kuehler (1974), Albert (1974), Harte and Stine (1977) was used with some additional modifications.

Raw milk, obtained from Michigan Milk Producers Association, was preheated to 130 - 135° F and homogenized at 1500 psi on the first stage and 500 psi on the second stage. The milk was pasteurized at 145° F for 30 minutes and cooled to 86°F. A frozen concentrated cheese starter culture (Hansen's Lab.,Inc., Milwaukee, WI) was then added to the cheese milk. When the acidity of the milk had increased 0.025 to 0.030, 116 g of Blue mold powder (*P. roqueforti*), obtained from Dairyland Food Lab, Inc., Waukosha, WI, and 60 ml of rennet per 1000 pounds of milk were added. After setting for approximately one hour, the curd was cut into 3/8 inch cubes. The curd was stirred gently 30 minutes after cutting and heated to 95° F for approximately one hour. The drained curd was placed on a fine mesh stainless steel screen in the ripening rooms which were maintained at a relative humidity of 90%. As the purpose of this project was to study the feasibility of

quick ripening Blue cheese by modified and gas flushing atmosphere packaging, many adaptations to the original method were attempted. These modifications dealt with salting times and amounts, temperature of curing, modified and gas flushing atmosphere packaging, type of packaging materials and physical treatment of Blue cheese curd such as gamma irradiation and microwave heating.

Statistical Analysis

Results of sensory evaluation were analyzed using a five-way analysis of variance (ANOVA) with interactions among random factors and fixed factors (Gill, 1978). The data were analyzed by the following statistical mixed model:

$$Y_{ijklmn} = u + A_i + B_j + AB_{ij} + C_m + D_l + AC_{im} + AD_{il} + BC_{jm} + BD_{jl} + CD_{ml} + F_n + AF_{in} + BF_{jn} + CF_{mn} + DF_{ln} + CDF_{mln} + E$$

Where Y_{ijklmn} = sampling variables

u = true mean of the Y distribution

A_i = replicates

B_j = panelists

C_m = packaging treatments

D_l = temperatures

F_n = storage times

E = random experimental error

Differences between means were analyzed for significance by Tukey's test (Gill, 1978).

RESULTS AND DISCUSSION

The Gas (O_2/CO_2) Requirements of P. roqueforti

The main objective of this research was to accelerate the ripening of Blue cheese curd by maintaining the optimum atmosphere over the packaged curd. Once the gas requirements of the mold and the optimum storage conditions are established, a package material with optimum barrier properties (gases and water vapor transmission) for the cheese can be selected.

Respiration Rate:

Figures 1 and 2 represent the respiration rate in Blue cheese curd, shown as CO_2 production (cc/kg/hr), salted with 3.5% salt concentration and stored at temperatures of 5°-20°C. The pattern of these curves appears to be similar to classical growth curve. Cheeses ripened at 20°C, coincided with first visible signs of mold growth, were faster than cheeses ripened at the other temperatures. The amount of CO_2 produced and the magnitude of the increase were directly related to the temperature employed in ripening. The maximum mold growth and CO_2 production were observed earlier at higher temperatures.

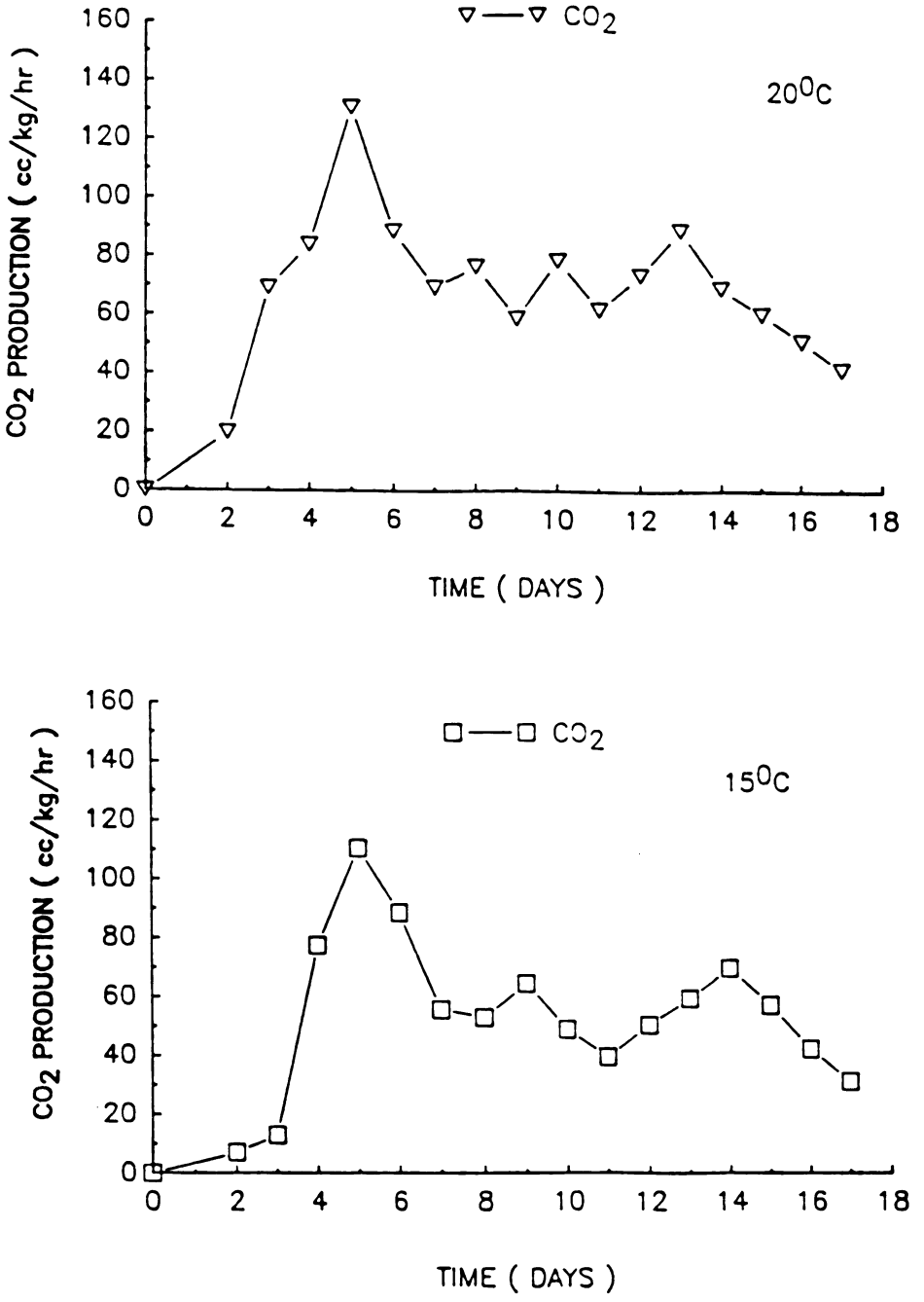


Figure 1. **Respiration rate in Blue cheese Curd, containing 3.5% NaCl.**

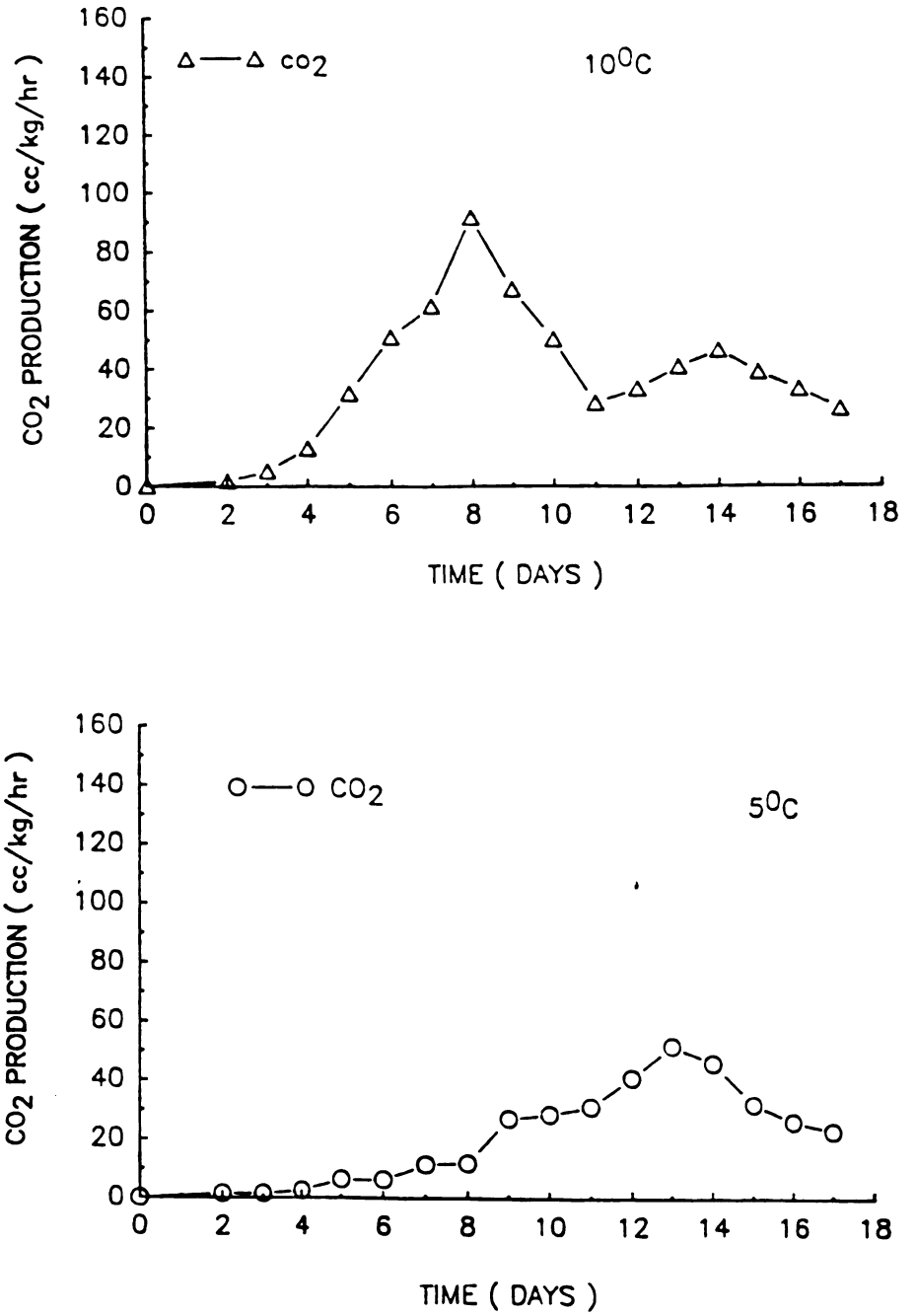


Figure 2. Respiration rate in Blue cheese curd, containing 3.5% NaCl.

The decrease in CO_2 production, presumably due to reduction of growth of P.roqueforti, was followed by an increase in CO_2 production for cheeses ripened at 20°C. This increase was also marked with signs of slime formation which was followed by development of orange and reddish color appeared on the cheese surface. The surface micro-flora most likely consisted predominantly of yeasts, several types of micrococci and Brevibacterium linens (Hartley and Jezeski, 1954). The excessive slime formation and discoloration for cheeses ripened at 20°C was detrimental to the flavor and color of the cheese. The discoloration was absent in cheeses ripened at 15°C and 10°C, however, slime formation was still slightly present.

Figures 3 and 4 represent the CO_2 production and O_2 consumption in Blue cheese curd containing 3.5% NaCL and ripened at different temperatures. The respiration quotient was almost constant throughout the ripening period. The amount of O_2 consumed was slightly higher than the amount of CO_2 produced (Table 2).

Subjective analyses on Blue cheese ripened at different temperatures were performed by the evaluators. Cheeses ripened at 20°C were heavily criticized in all areas. At this ripening temperature, excessive mold growth occurred which resulted in a sticky, gummy texture, and moldy flavor. Cheeses ripened at 5°C were criticized for lacking in mold

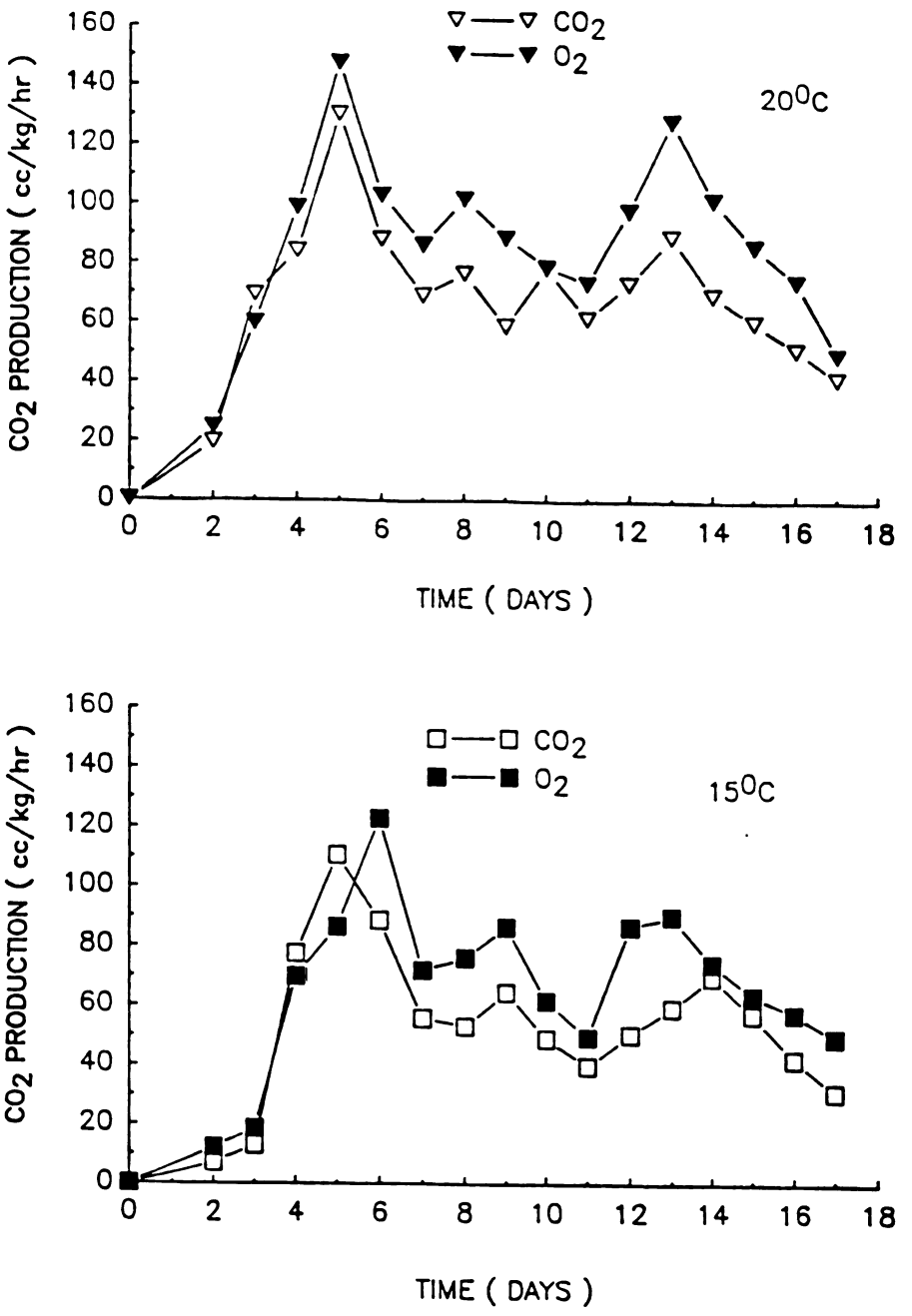


Figure 3. CO_2 production and O_2 consumption in Blue cheese curd, containing 3.5% NaCl.

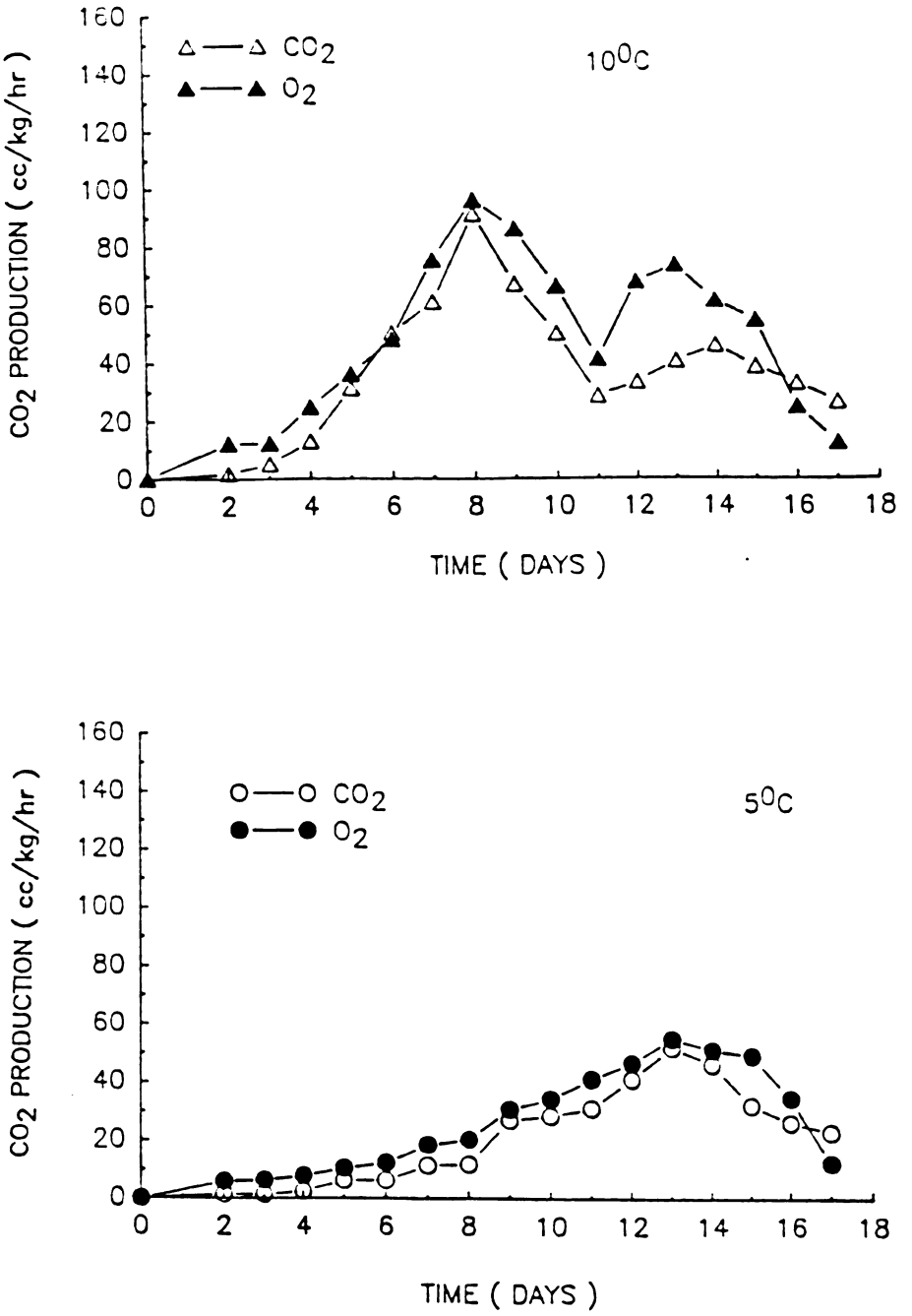


Figure 4. CO_2 production and O_2 consumption in Blue cheese curd, containing 3.5% NaCl.

growth and having very mild flavor. Cheeses ripened at 10°C and 15°C were considered good but the major fault was a mild flavor.

Table 2.

Maximum CO_2 production, O_2 consumption and Respiration Quotient in Blue cheese curd ripened at different temperatures.

Temperature (°C)	Time* (Days)	cc/kg/hr		Respiration Quotient (CO_2 : O_2)
		CO_2	O_2	
5	13	52.45	55.68	1 : 1.06
10	8	91.39	96.20	1 : 1.05
15	6	110.39	122.66	1 : 1.11
20	5	130.70	148.00	1 : 1.13

* Time required for ideal mold growth.

Oxygen Depletion:

Oxygen concentrations of 76% and more have been shown to demonstrate a definite inhibitory effect on certain molds (Karsner and Saphir, 1926). In the present study, the effect of oxygen concentration (21% and 100% O_2) on the growth of P.roqueforti was investigated using a closed system with a CO_2-free atmosphere.

Figures 5 and 6 represent the oxygen depletion at initial oxygen concentrations of 21% and 100%, respectively, in Blue cheese curd ripened at various temperatures. In an oxygen atmosphere of 21%, complete depletion of oxygen was

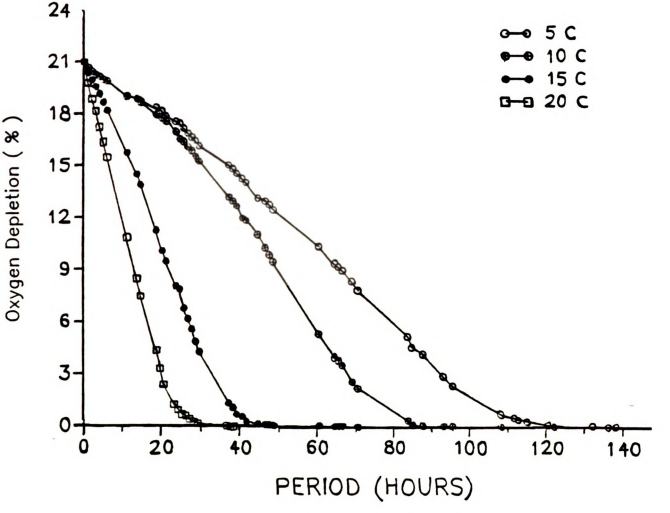


Figure 5. O_2 Depletion in Blue cheese curd ripened at various temperatures in 21% O_2 atmosphere.

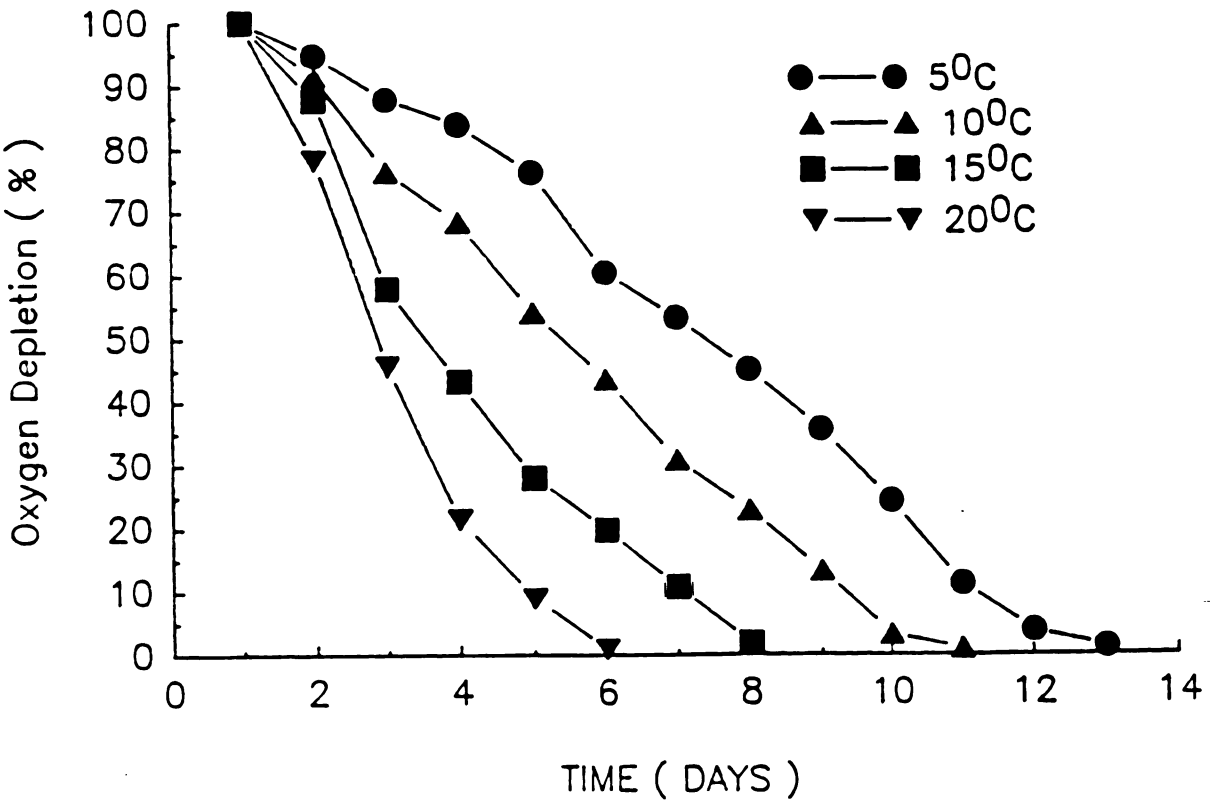


Figure 6. O_2 Depletion in Blue cheese curd ripened at various temperatures in 100% O_2 atmosphere.

reached between 1-5 days of ripening. No indication of mold growth was observed for any of the temperatures used. In an oxygen atmosphere of 100%, good mold growth was observed on days 4, 6, 8, and 11 of ripening at 20°, 15°, 10°, and 5°C, respectively. Theses data are in agreement with Golding (1940 II) and supported the contention that the absence of oxygen was a limiting factor in the growth of P.roqueforti in the Blue cheese. It also appeared that an initial oxygen concentration of 100% did not inhibit the growth of P.roqueforti.

Selection of Product-Package Parameters:

Based on the data obtained, the following parameters were selected to investigate the possibility of ripening Blue cheese curd in modified and gas flushed atmosphere packaging:

1. Temperatures of 10° and 15°C were shown by the sensory analysis to be ideal for cheese ripening. The cheese improved in flavor as the temperature of ripening was lowered. Lowering the ripening temperature probably retarded contaminating organisms more than retarding the desired fermentation caused by P.roqueforti (Kuehler, 1974).
2. Flexible pouches of material, Low Density Polyethylene (LDPE), were selected for the modified atmosphere

packaging. The low water vapor transmission rate (Table 3) and the high gas permeability rate of LDPE (as was determined experimentally) were considered critical to allow the cheese to ripen. Other important functional characteristics of LDPE, such as flexibility tasteless, odorless, and chemically inert (Hanlon, 1983) were also considered. The transparency of LDPE permits the ripening process to be monitored without the need to open the packages. The oxygen permeability rate of 1 mil LDPE (Dow USA, Midland,MI) was 1920 cc/m^2/24hrs., which was comparable to the oxygen requirement of Blue cheese curd as determined by the respiration rate.

Table 3.

Water Vapor Transmission of LDPE and Laminate (PE/Nylon) pouches at various temperatures.

Temperature (°C)	gram/100 in^2/24 hr	
	LDPE	PE/Nylon
5	0.61	0.41
10	0.88	0.66
15	1.19	0.87

3. Pre-formed pouches made from a good barrier film laminate (Nylon/PE), obtained from Koch Co., Kansas

City, MO., were used for the gas flushed atmosphere packaging. The oxygen permeability rate of the laminate (3.0 mil) was 0.041 cc/m^2/24hrs. The water vapor transmission rates are shown in Table 3. The possibility of ripening Blue cheese curd by flushing the pouches with 100% initial oxygen concentration was then explored.

4. The salt concentrations (3.5% and 4.5%) were added to the drained Blue cheese curd on the first day. The following day, the Blue cheese curd was then packaged (100 g/pkg) into the selected LDPE and Laminate pouches (6" x 8.5"). The pouches were then sealed with an impulse heat sealer. A portion of the cheese was maintained as a control (unpackaged). All initial analytical data were performed as described in the Experimental Section.

Changes in pH During Ripening

The pH of Cheese is considered important because it could influence the activity of enzyme systems during the ripening period. Therefore, the changes in pH during ripening of Quick Ripened (QR) Blue cheese were determined. A comminuted, representative sample of cheese was analyzed each day of the ripening period. Fresh curd exhibited a pH value of approximately 5.64 prior to being placed in the

curing room. The hydrogen ion concentrations reached a maximum at about pH 5.19 - 5.38 on the second day of ripening. This was followed by a rapid increase to 6.03 - 6.98 which generally coincided with the first visible signs of mold growth and occurred on the fourth, fifth and/or sixth days of ripening. At the end of ripening, the pH stabilized or in some cases decreased slightly to approximately 5.46 - 6.49 for the remainder of ripening. Varying treatments such as ripening temperature and packaging procedure were responsible for the wide differences found in pH value during ripening.

Figures 7 and 8 represent the change in pH during ripening of QR Blue cheese curd at 10°C and 15°C, respectively. These cheeses showed similar trends in pH during ripening. The initial increase in acidity is due to the production of lactic acid by Streptococcus lactis starter culture (Morris, 1963). The maximum acidity was not reached until 48 hours after manufacture. The low production and slow acid development might be attributable to the relatively low amount of starter used (1%), and the early salting time which may retard the starter activity. Kuehler (1974) and Harte and Stine (1977) reported that the hydrogen ion concentration reached a maximum at about 4.66 and 4.91, respectively, for QR Blue cheese ripened at 17°C and salted on days 4, 5 and 6. The authors attributed the

slow acid development during the initial stages of ripening to the low amount of starter used (1%) as opposed to the generally accepted commercial amount of 2 - 4% of starter. Slower acid development was noted also by Morris and Jezeski (1953) when 0.75 % starter was used. A gradual increase in pH occurred 48 to 96 hours, after manufacture before a rapid increase in pH was noted. Foster et al.(1961) and Harte (1974) suggested lactic acid degradation by P.roqueforti as a reason for the initial rise in pH. The maximum increase in pH occurred on the 6th and 7th days of ripening and may be attributed to proteolysis by the protease enzymes associated with P.roqueforti. Lane and Hammer (1938), Morris et al. (1963), Kuehler (1974) and Harte and Stine (1977) noted that pH increased as proteolysis increased in the ripening cheese. The final stabilization or the slight decrease in pH values near the end of ripening period may be explained by the increasing levels of free fatty acids liberated by the mold lipase system. Coulter et al. (1938) and Morris et al. (1963) noted a slow decrease in pH to 5.7 at 4 to 9 months in Blue cheese and attributed this decrease in pH to an accumulation of free fatty acids in the cheese. A decrease in proteolysis could also have contributed to the stabilizing effect. Modler et al. (1974) noted that the activity of the protease(s), isolated from P.roqueforti, dropped off rapidly below 30°C.

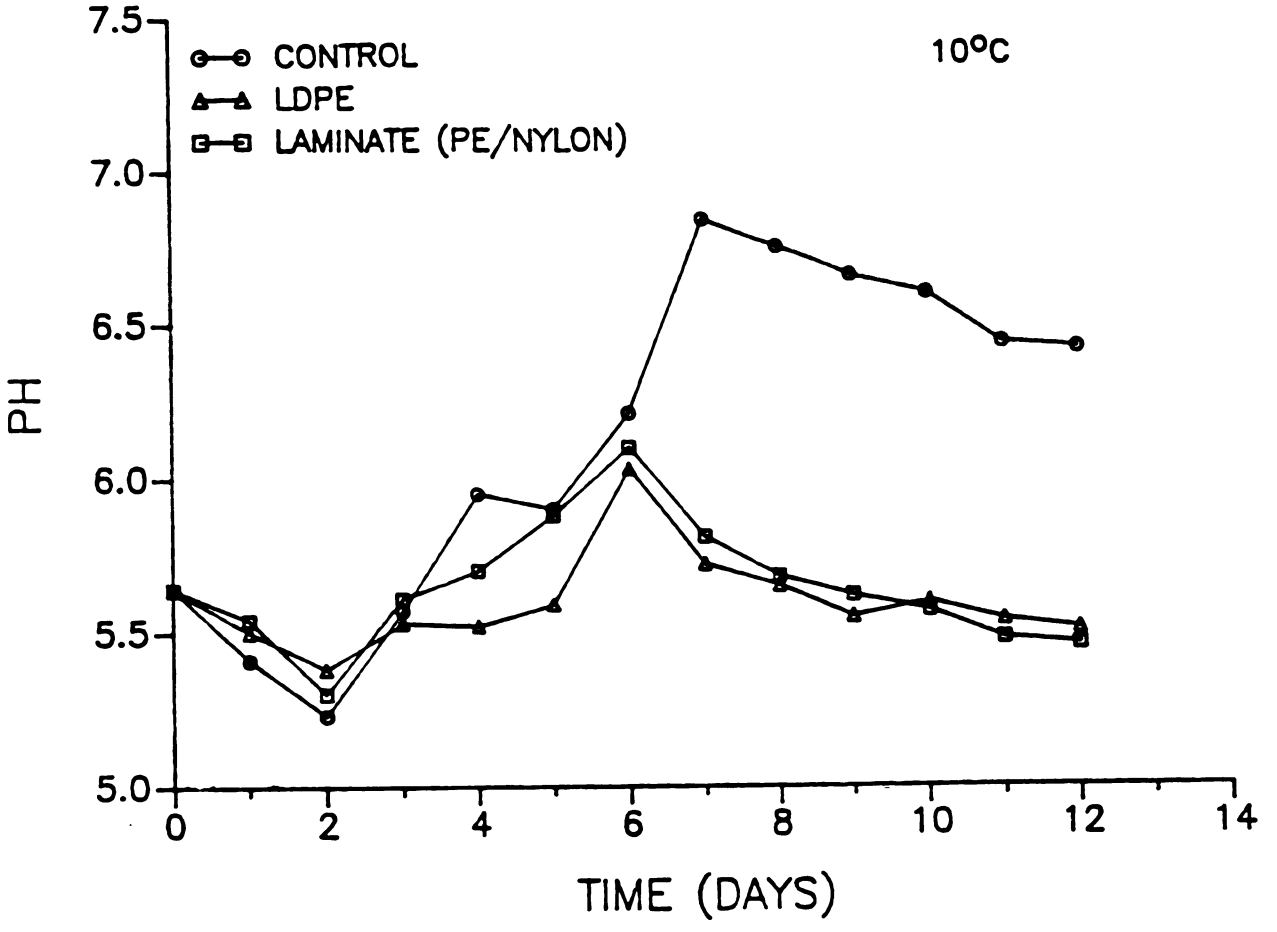


Figure 7. Change in pH during ripening of quick ripened Blue cheese curd at 10°C in modified and gas flushed atmosphere packaging.

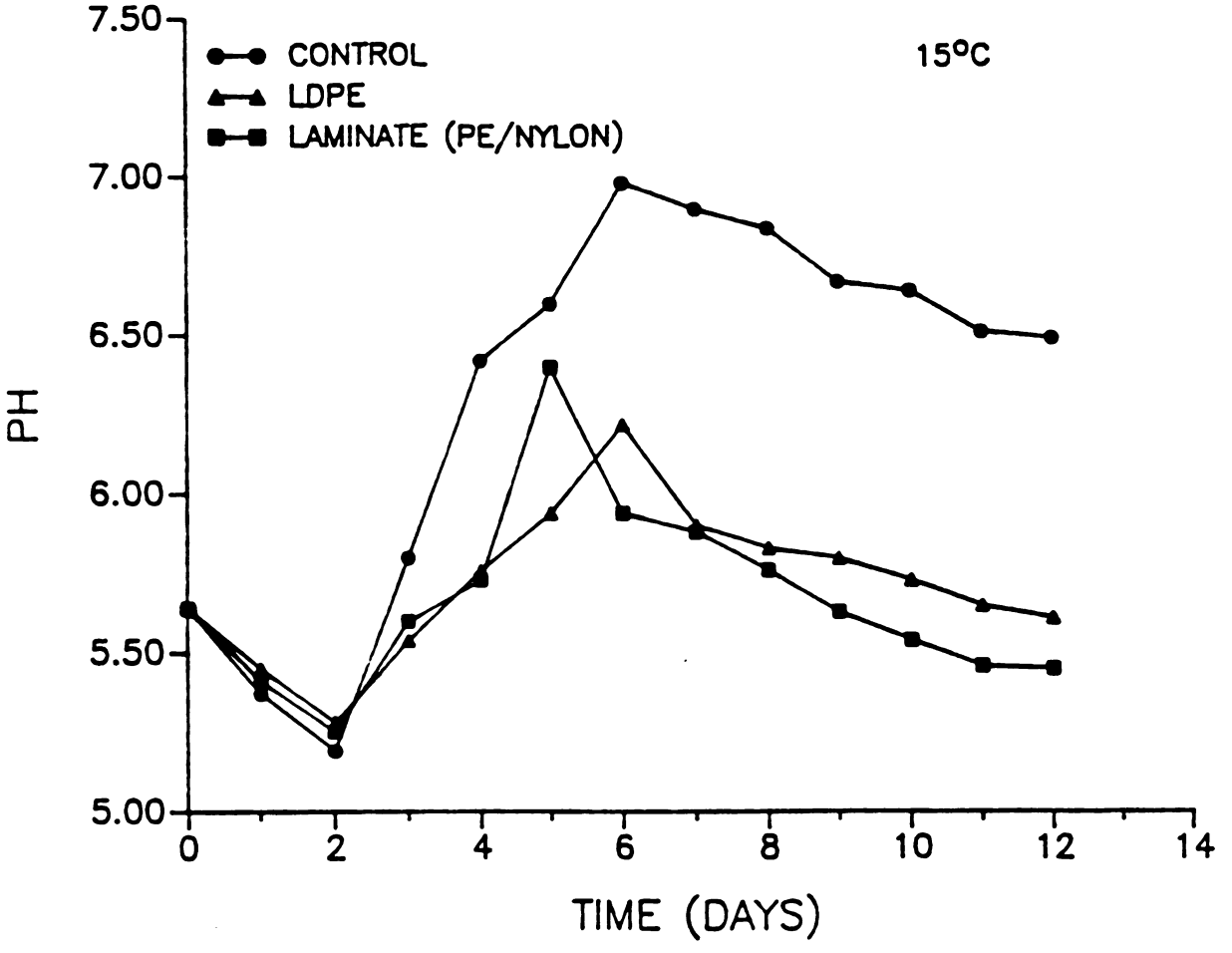


Figure 8. Change in pH during ripening of quick ripened Blue cheese curd at 15°C in modified and gas flushed atmosphere packaging.

The most obvious effect of temperature on cheese ripening was that as temperature was increased, there was a decrease in the time required for ripening (Kuehler, 1974). The 22°C ripened cheese reached a minimum pH on the first day and 6 days were required for the 9°C cheese to obtain a comparable hydrogen ion concentration (Kuehler, 1974).

Figure 9 reveals the effect of temperature on changes in pH during ripening. The rapidity of change in pH and magnitude of these changes were directly related to the temperature employed in ripening. A significant increase ($P < 0.05$) was observed between 3-6 days of ripening. This was possibly due to increased activity of the starter cultures at the elevated temperatures used. The rapid increase in pH, which was attributed to proteolysis, at 15°C was delayed and became more gradual as the temperature was lowered to 10°C. The control cheese at 15°C reached a maximum pH of 6.98 on the 6th day, while a pH value of 6.84 was attained on the 7th day of ripening at 10°C. There was no significant differences ($P < 0.05$) between cheeses ripened at 10°C and 15°C for the remainder of the ripening period.

Figure 7 demonstrates the effect of modified and gas flushed atmosphere packaging on the change in pH during ripening as opposed to the control cheese. These cheeses showed varying pH values. A gradual increase in pH was

noted for modified atmosphere packaged cheese as opposed to the rapid increase in pH for the control cheese. Cheeses in modified and gas flushed atmosphere packaging had a maximum pH value of 6.03 and 6.19, respectively, as opposed to a value of 6.84 for the control cheese at the same temperature (10°C). The considerable variation in pH values between the control and the cheeses in modified and gas flushed atmosphere packaging is likely due to the reduced O_2 level and increased CO_2 concentration in the package headspace. The low O_2 content inside the pouches can lead to a decrease in mold growth and activity (Golding, 1940 II). The adsorption of CO_2 on the cheese surface and partial dissociation of carbonic acid may lead to increase in hydrogen ion concentration. The solubility of CO_2 increases as the temperature decreases (Ogrydziak and Brown, 1982). This may explain the slight increase in hydrogen ion concentration at 10°C as compared to 15°C. Another factor that could be considered and may have had an effect on the pH of the cheese is the treatment given to milk before, during and after its manufacture into Blue cheese.

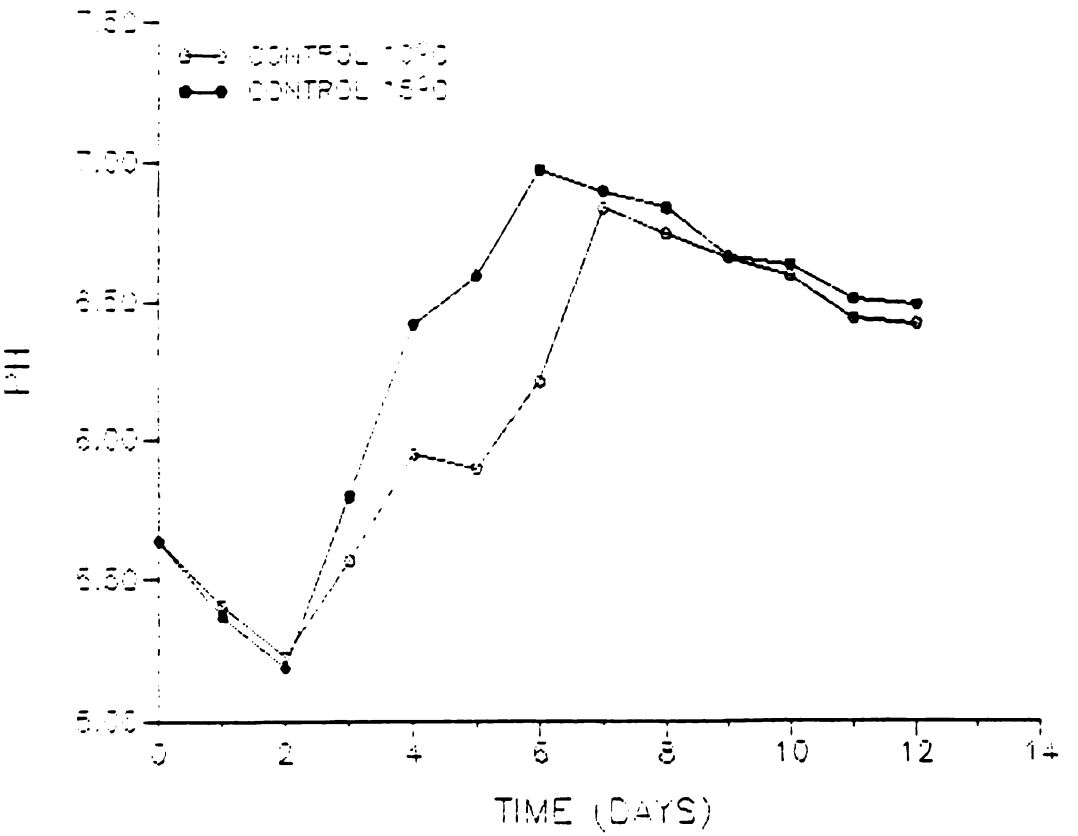


Figure 9. The effect of temperature on pH during ripening of quick ripened Blue cheese curd.

Changes in Water Soluble Nitrogen During Ripening:

Water soluble nitrogen values were used as an indicator of proteolysis occurring during the ripening of QR Blue cheese. Morris et al. (1963) used tyrosine liberation, while Kuehler (1974) used NPN values as an indicator for protein degradation. Water soluble nitrogen was defined in this study as the percent of total protein soluble after precipitation of casein at pH 4.6 using the sodium citrate-hydrochloric acid procedure (Vakaleris and Price, 1959).

Figures 10 and 11 represent the changes in water soluble nitrogen during ripening of QR Blue cheese curd at 10°C and 15°C, respectively. The curves obtained were similar in all preparations of Blue cheese curd. The gradual increase in water soluble nitrogen was observed in the early part of ripening. This was followed by a rapid increase in the amount of soluble protein liberated from the cheese. A slight increase in soluble protein was the characteristic of the last days of ripening in all preparations of Blue cheese curd. Generally, 32 - 43% of the total protein was present in the soluble form at the end of ripening. Keuhler (1974) reported that 45 - 60% of the total protein in QR Blue cheese was present in the soluble form following precipitation with 12% TCA. Jolly and Kosikowski (1975) reported that soluble nitrogenous compounds comprised about 25% of the total nitrogen in

ripened Blue cheese and 34% of cheese made with added lipase. Godinho and Fox (1982) reported that concentration of NPN reached a maximum of 25 -35% of the total nitrogen in Blue cheese. The increase in soluble protein early in the ripening period was likely due to proteolysis resulting from the starter culture and rennet (Kuehler, 1974). The rapid increase in soluble protein occurred 48 - 72 hours after manufacture and probably was due almost exclusively to the protease system of P.roqueforti. The rapid increase in soluble protein also coincided with the rapid increase in pH and the first appearance of mold growth. These data are in agreement with Morris et al.(1951), Kuehler (1974), Trieu-Cuot and Gripon (1983) and supported the contention that the rapid increase in pH was due to proteolysis. Toward the end of ripening there were slight increases in soluble protein and this level was maintained during an extended ripening period was used. Several factors may have attributed to the slight increase. Imamura and Kataoka (1966) demonstrated that volatile fatty acids formed by action of P.roqueforti lipase were inhibitory to the mold protease.

Variation in treatments such as salting time and concentration, ripening temperature and packaging procedure were responsible for the wide differences found in soluble protein values.

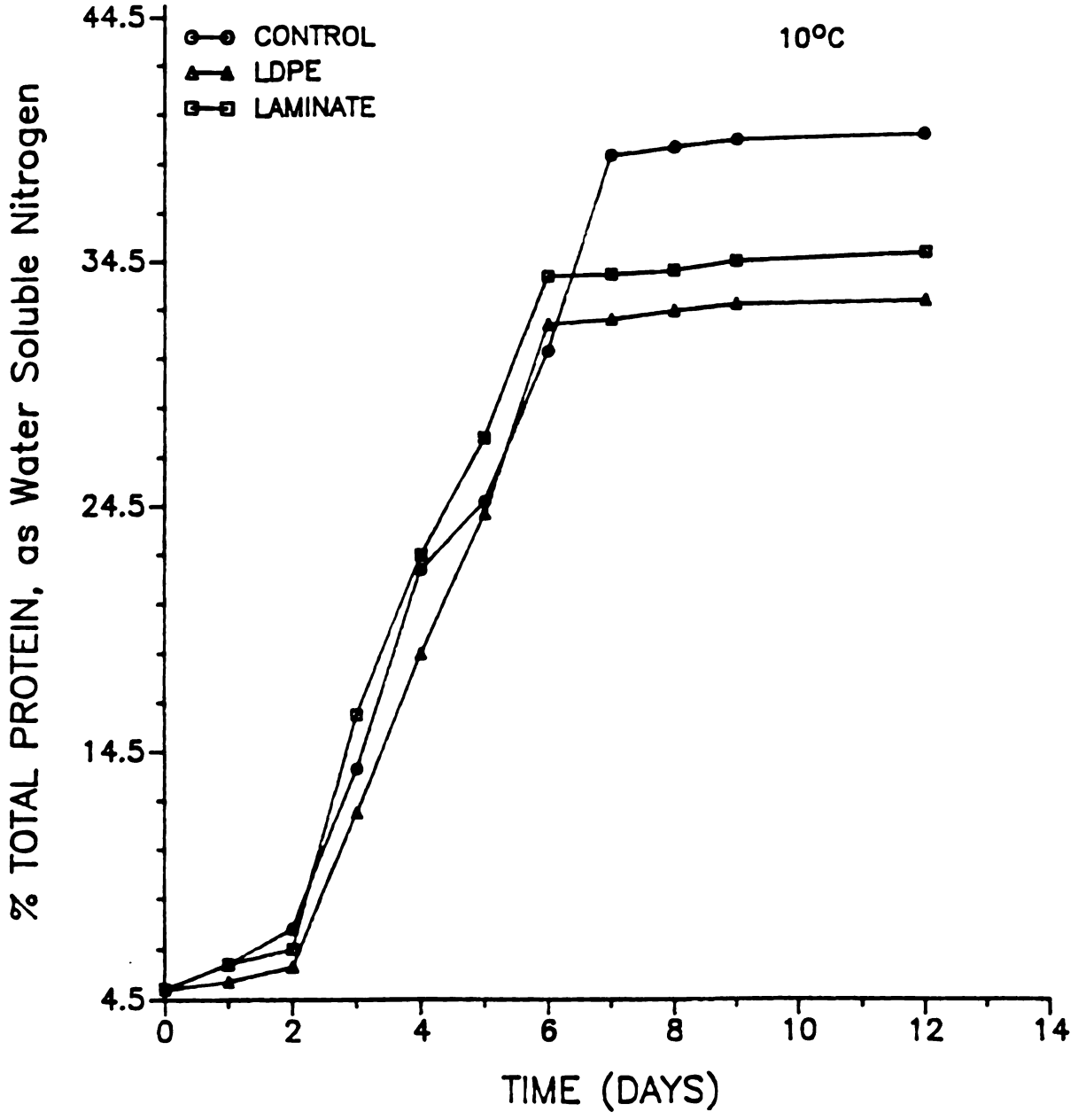


Figure 10. Change in water-soluble nitrogen of quick ripened Blue cheese curd at 10°C.

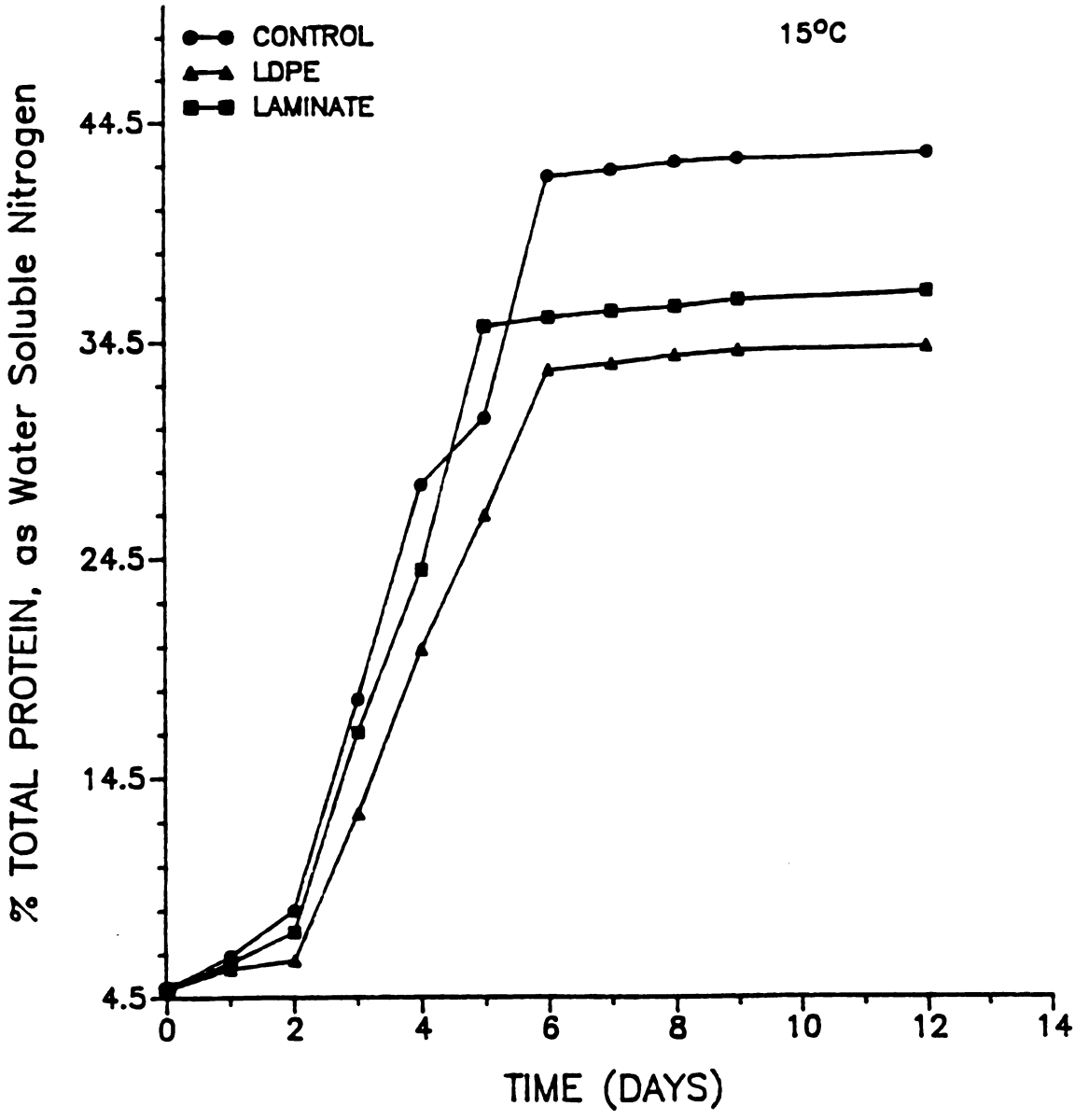


Figure 11. Change in water-soluble nitrogen of quick ripened Blue cheese curd at 15°C.

Figure 12 represents the effect of salt concentration on water soluble nitrogen of QR Blue cheese curd at 10°C. The concentration of soluble protein reached a maximum of about 30.8%, at a salt concentration of 4.5%, on the 9th day of ripening while at 3.5% salt concentration the maximum increase was attained on the 7th day and reached 38.5%. A significant decrease ($P < 0.05$) in soluble protein resulted as the salt concentration increased from 3.5 to 4.5%. The early application of salt and the increasing salt concentration appeared to retard proteolysis. Kuehler (1974) reported that proteolysis was retarded when at least 50% of the salt was added prior to the fourth day of ripening. Gripon and Hermier (1974) demonstrated the inhibitory effect of sodium chloride on the extracellular protease. Kinsella and Hwang (1976) and Godinho and Fox (1982) noted the same effect. The effect of temperature on the changes in soluble protein is shown in Figure 13. The amount of soluble protein increased rapidly at 15°C and reached a maximum of about 42.1% on the 6th day of ripening while at 10°C the maximum increase was attained on the 7th day and was 38.5%. A significant increase ($P < 0.05$) was obtained when the temperature was increased to 15°C. This level of increase was maintained throughout the ripening period. Kuehler (1974) noted the same effect of temperature on the changes in NPN of QR Blue cheese.

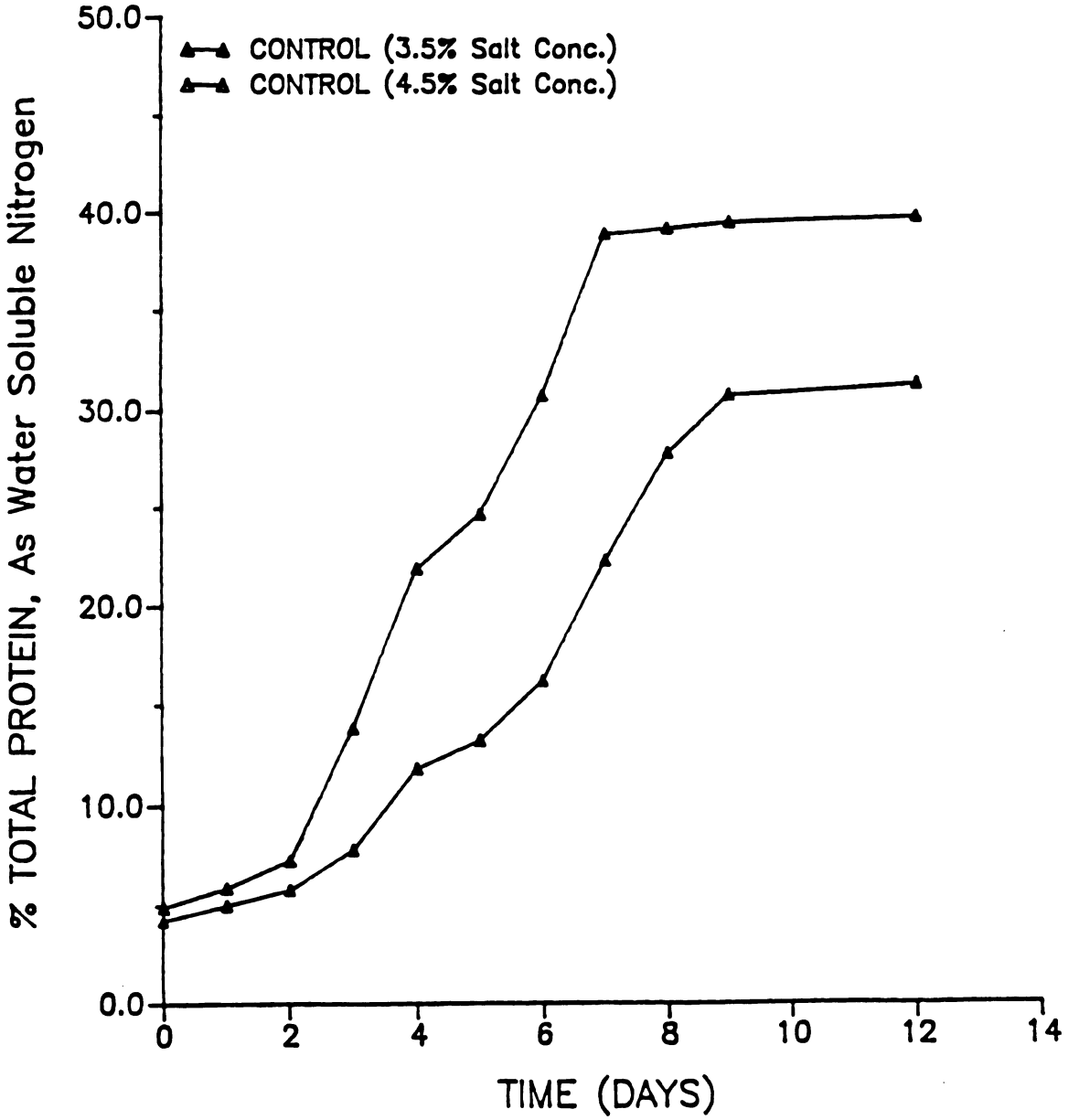


Figure 12. The effect of salt concentration on water-soluble nitrogen of quick ripened Blue cheese curd at 10°C.

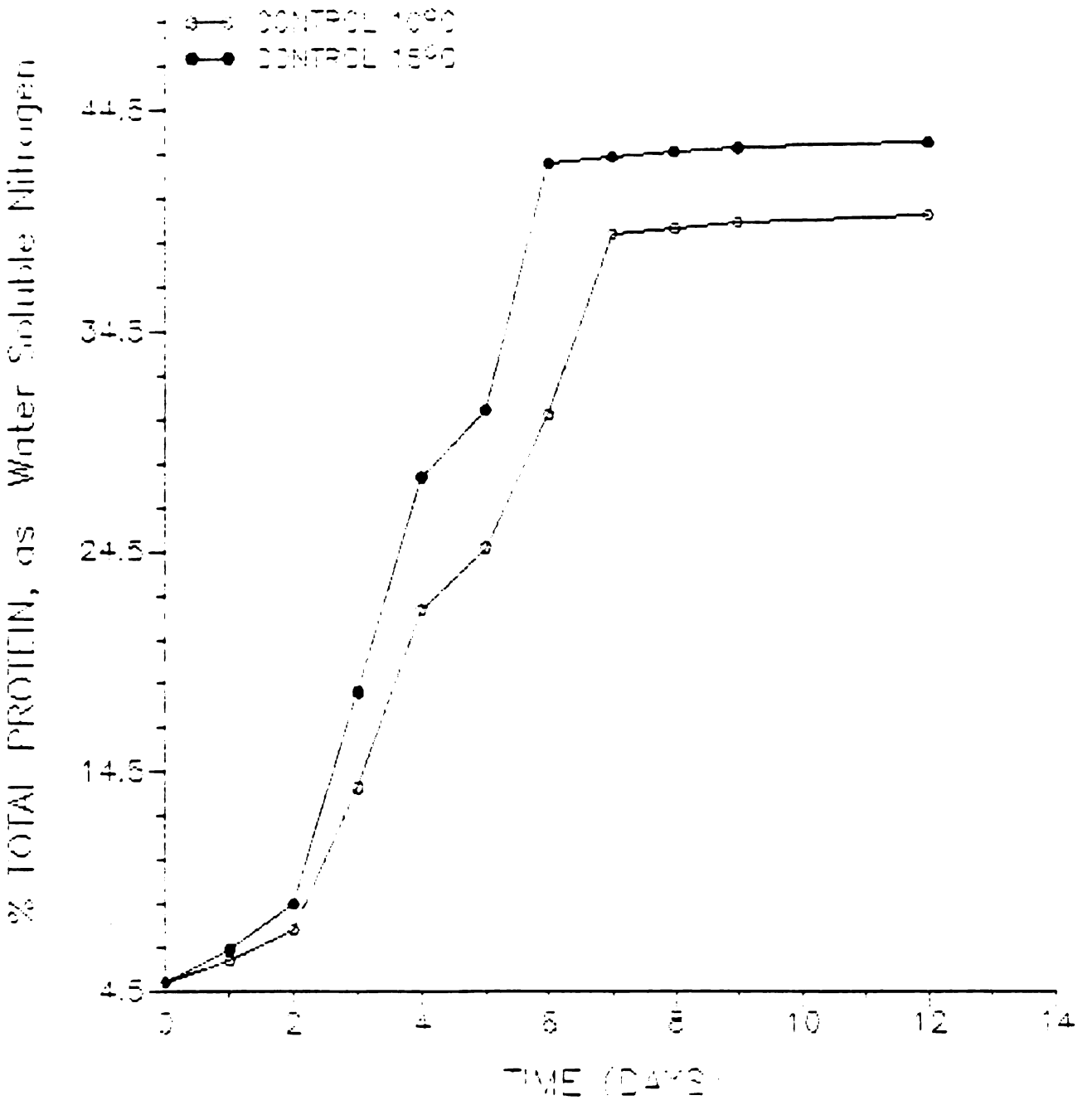


Figure 13. The effect of temperature on water-soluble nitrogen of quick ripened Blue cheese curd.

Figures 10 and 11 show the effect of modified and gas flushed atmosphere packaging on the water-soluble nitrogen. The plots for these cheeses were similar but varied in the total amount of soluble protein. Cheeses in the modified and gas flushed atmosphere packaging reached a maximum amount of 31.9% and 33.9%, respectively, on the 5th and 6th day of ripening at 10°C. A slight increase was noted during the remainder of the ripening period. A significant decrease ($P < 0.05$) in the amount of soluble protein was observed as a result of atmospheric modification (21 - 24% decrease in modified atmosphere; 14 - 19% decrease in gas flushed) as compared to the control cheese. This decrease may be attributed to the reduced oxygen atmosphere and/or increase of carbon dioxide concentration inside the pouches which may have retarded mold growth, hence production of proteolytic enzymes capable of hydrolyzing the cheese proteins was reduced. There was no significant increase ($P < 0.05$) in soluble protein for cheeses in modified atmosphere as compared to gas flushed atmosphere at the same or different temperatures. The slight increase observed is likely to occur due to the increased activity of proteolytic enzymes and/or increased gas permeation through microscopic pores and pinholes of the package material as the temperature increased. Karel (1974) reported that, for a given temperature and above this temperature (or temperature

range), plastics packaging material is more permeable. The amount of soluble protein in gas flushed cheeses was slightly higher throughout the ripening period and the maximum amount obtained one day sooner than in the modified atmosphere cheeses at 15°C. This may be attributed to a higher oxygen level in the packages early in the ripening period and/or the lower carbon dioxide concentration in gas flushed atmosphere cheeses. King and Nagel (1975) concluded that a high concentration of CO_2 would inhibit the metabolic activity of the microbial flora. This inhibitory effect is increased at a lower temperature of 5°C (Finne, 1982).

In general, proteolysis was inversely related to salt concentration in the cheese and directly proportional to the temperature of ripening. The packaging procedures had profound effect on QR Blue cheese by controlling excessive proteolysis which otherwise may have resulted in a bitter after taste.

Gas Headspace Analysis

The ambient conditions in the storage rooms during the experiments were ca. 20.7% oxygen and 0.05% carbon dioxide.

A. Modified Atmosphere Packages

The changes in headspace CO_2 and O_2 quantities in the LDPE pouches were monitored during the ripening period

at 10°C and 15°C (Figure 14). A rapid decrease in O_2 concentration was observed in the package headspace before an equilibrium level of 2 - 3% was reached at 6 - 7 days of ripening. This rapid decrease in O_2 concentration coincided with a rapid increase in CO_2 production and occurred at 4-6 days of ripening. This was probably due to the respiratory activity of P.roqueforti in the packaged cheese. The rapid increase in CO_2 was followed by a gradual decrease before equilibrium levels of 5 - 6% were reached at 8 - 9 days ripening. This gradual decrease was likely due to a decrease in respiratory activity as a result of low O_2 concentration inside the packages. Golding (1940.II) reported that O_2 concentration below 4.2% resulted in significant reduction in P.roqueforti growth. The equilibrium levels for O_2 and CO_2 were maintained throughout the remainder of ripening period and were in good agreement with the permeability rate of the film. The packages headspace gases (CO_2 and O_2) at equilibrium did not increase significantly ($P < 0.05$) as the temperature increased to 15°C. This would suggest that, at the equilibrium within the package, the respiration rate (CO_2 production or O_2 consumption) of Blue cheese curd would be equal to the package permeation rate. Tomkins (1962) studied the

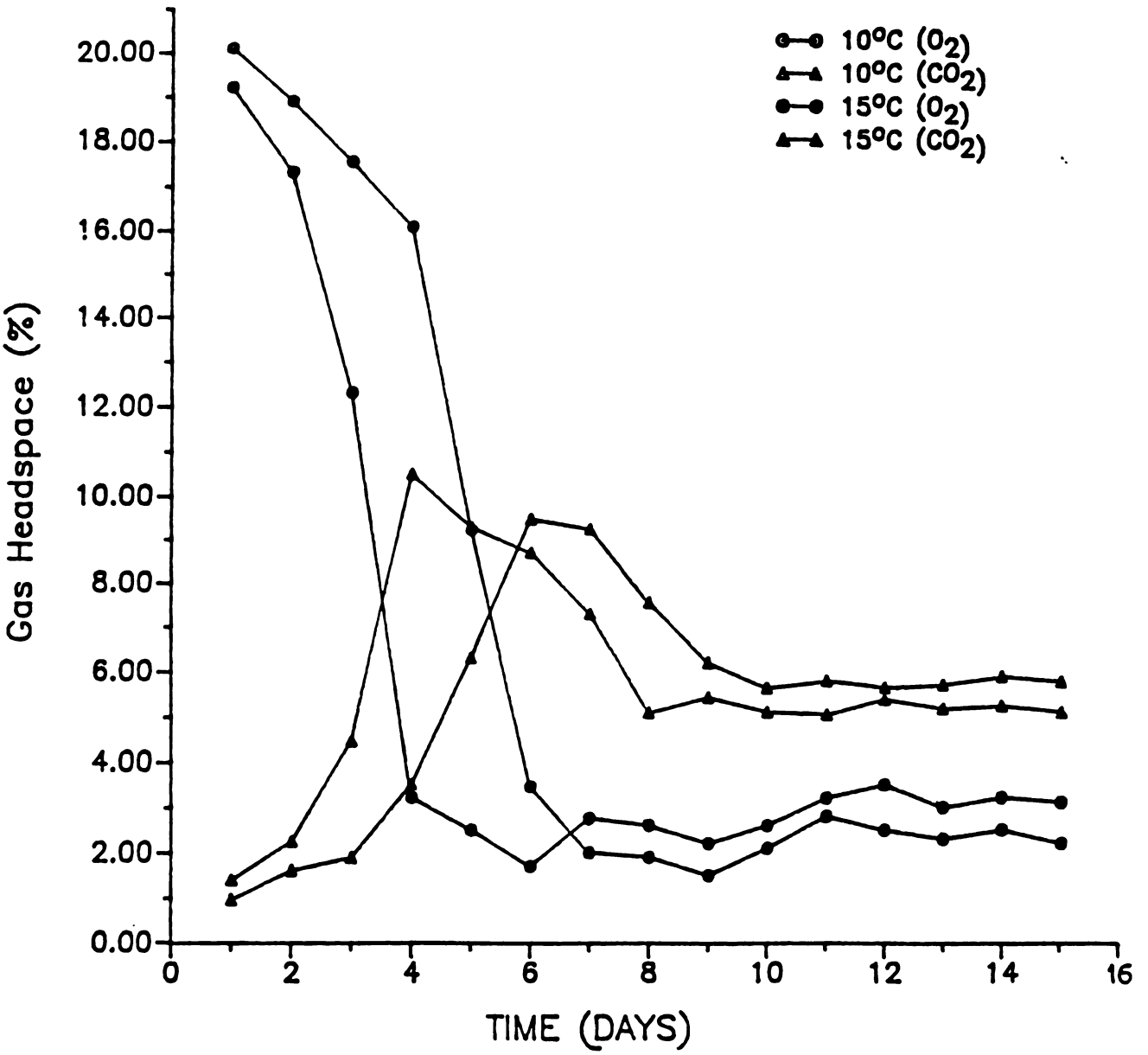


Figure 14. Change in package headspace gas concentration during ripening of Blue cheese curd in modified atmosphere packaging.

dynamics of a polymeric film package and cited that, at the equilibrium, the respiration rate was assumed to be equal to the permeation rate. The equilibrium levels of oxygen in the packages were slightly higher at 15°C (2.80% ± 0.32) than that at 10°C (2.40% ± 0.24), while CO_2 levels were slightly lower (5.30% ± 0.32) at 15°C than that at 10°C (5.80% ± 0.35). This was most likely due to the increased film permeation as the temperature of ripening increased and/or the film exposure to high humidity. Exposure of certain films to high humidity enhances the mobility of the polymer chain and results in increasing the permeability to all gases (Karel, 1974; Contreras-Medellin, 1980). Increased equilibrium O_2 levels and decreased CO_2 levels in LDPE packages as the storage temperature was increased from 20°C to 25°C were demonstrated by Prince (1983). Maximum CO_2 production (10.50 %) was attained on the 4th day of ripening at 15°C while at 10°C, the maximum of 9.48 % was reached on the 6th day of ripening. The rapidity of attainment at the high temperature was likely due to an increased respiratory activity in the curd as a result of increased mold growth at higher temperature.

B. Gas Flushed Atmosphere Packages

The oxygen depletion during ripening of QR Blue cheese curd at 10°C and 15°C in Laminate (PE / Nylon)

pouches was monitored (Figure 15). Small bags (LDPE) containing calcium hydroxide (ca. 5.0 g) were inserted inside the pouches to absorb CO_2 in order to eliminate its inhibitory effect. The calcium hydroxide effectively maintained CO_2 levels below 0.5 % inside the pouches. A rapid decrease in oxygen concentration was observed before an equilibrium oxygen levels was reached. Maximum consumption of oxygen occurred between 4 - 6 days and 2 - 4 days of ripening at 10°C and 15°C, respectively, and coincided with visible mold growth. The rapidity of oxygen depletion at 15°C was attributed to the increased respiratory activity in the pouches as a result of P.roqueforti growth. An oxygen equilibrium was attained at 8 - 9 days of ripening for both temperatures and was maintained thereafter. At equilibrium, there was no significant difference ($P < 0.05$) between cheeses ripened at 10°C and 15°C.

The data herein reported show clearly that the O_2/CO_2 requirements of P.roqueforti are functions of both gas concentration and temperature and that mutually dependent relationship exists between respiration and permeation within the Blue cheese curd packaging system.

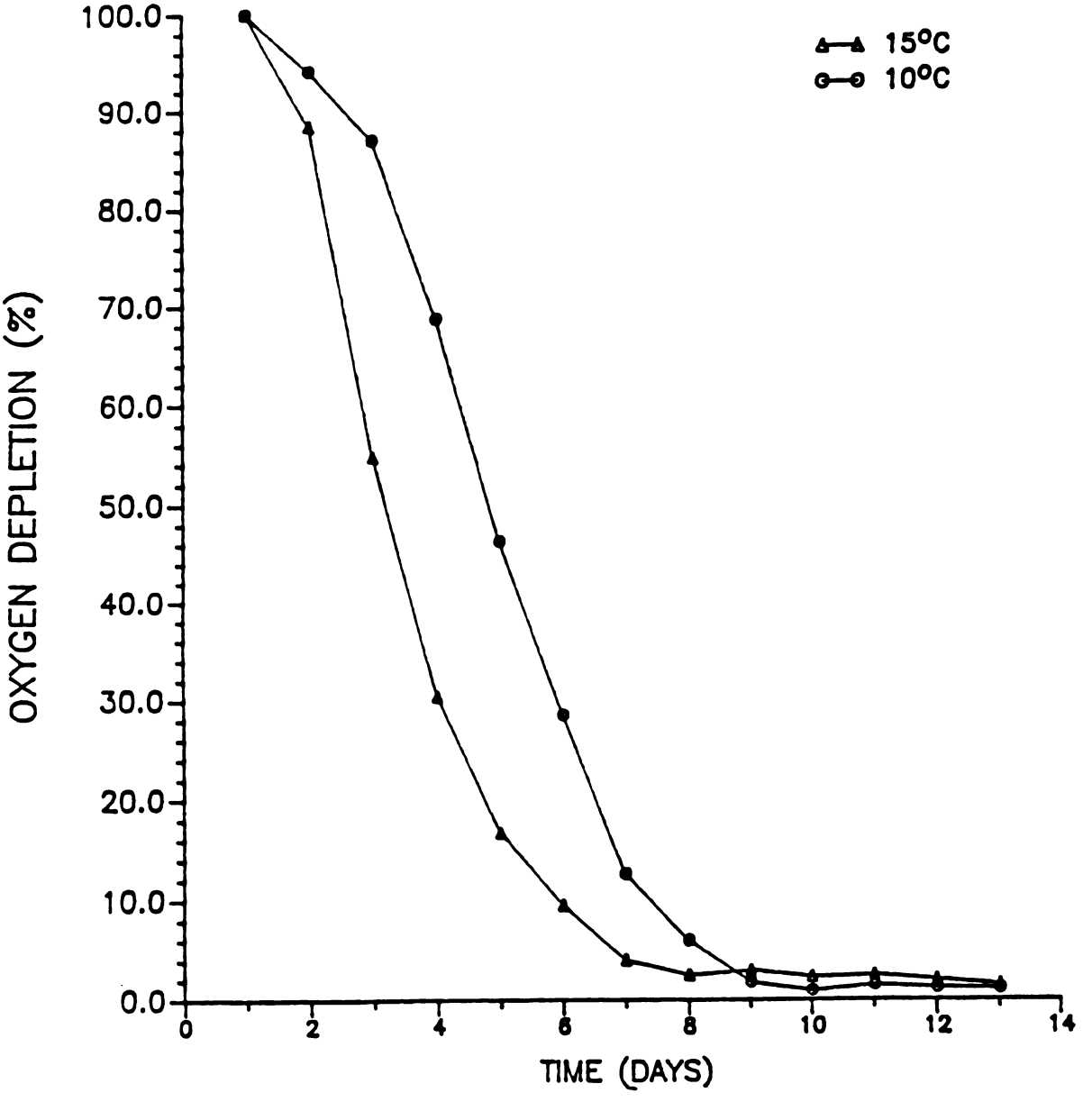


Figure 15. Oxygen depletion during ripening of quick ripened Blue cheese curd at 10°C and 15°C in gas flushed atmosphere packaging.

Change in Curd Moisture Content During Ripening:

The amount of moisture in the curd determined in triplicate in order to observe moisture loss during ripening and storage. The results are shown in Table 4. The initial moisture content was dependent upon the manufacturing process. Failure of the starter culture to develop a sufficient acidity during manufacture resulted in retention of more water in the curd. Cooking the curd for one hour at 95° - 100°F resulted in faster drainage and strengthened the curd particles. The cooking did not appear to adversely affect the quality of the cheese (Harte, 1974).

The initial moisture content, prior to packaging was 50.34% and decreased rapidly during two weeks of ripening at 10°C and 15°C. The rate of moisture loss varied with the temperature and packaging procedure. During two weeks of ripening at 10°C and 15°C,respectively, 8-10% moisture loss occurred in the control (unpackaged) cheeses, while 3-4% and 4-5% moisture loss was observed for cheeses in gas flushed and modified atmosphere packaging, respectively. Moisture appeared to be drawn from the inner to the outer regions of the cheese curd possibly due to osmotic pressure effects arising from higher salt concentrations on the surface (Godinho and Fox, 1981a).

Table 4.

Moisture Content* (%) of Blue cheese curd salted on day one (3.5%) and quick ripened at 10°C or 15°C over 95% RH for 2 weeks then stored at 5°C/95% RH for additional 2 weeks.

Time (Weeks)	Modified Atmosphere (LDPE)		gas flushed Laminate (PE/Nylon)		Control (Unpackaged)	
	10°C	15°C	10°C	15°C	10°C	15°C
Initial (prior to packaging)	50.34	50.34	50.34	50.34	50.34	50.34
1	47.91	47.21	48.81	48.21	46.40	44.70
2	46.30	45.92	47.19	46.29	43.31	41.80
3	45.96	45.50	46.80	45.95	42.85	41.45
4	45.71	45.37	46.65	45.70	42.61	41.21

* Triplicate analysis.

Although the moisture contents varied, the rate of moisture loss was quite similar. Moisture loss was faster and higher in the cheeses ripened at the higher temperature as a result of evaporation and the utilization of moisture by P. roqueforti. The same effect of temperature on moisture loss was found by Harte (1974) and Dokhani (1978). The variation in percent moisture among the unpackaged and packaged cheeses was a result of the water vapor barrier of the packaging materials. The permeability rate of the films allowed more moisture to remain in the packages, which resulted in softer cheeses. The increased moisture loss of packaged cheese at the higher ripening temperature was also partially due to increased water permeation rate of the

packaging materials at the higher temperature. During storage of curd samples for two weeks at 5°C/95% RH, less than 1% moisture loss was observed. This occurred presumably as a result of continued inward diffusion of salt (Godinho and Fox, 1981a) which could stabilize the osmotic pressure and also as a result of the lower temperature employed (Contreras-Medellin, 1980).

The effect of temperature and salt concentration on the moisture content of QR Blue cheese during ripening is shown in Table 5. The moisture difference reached approximately 10% as a result of employing higher ripening temperature (QR_1 and QR_2). The final moisture content was directly related to the salt content, possibly because of improved moisture holding capacity of the higher salt concentration (Godinho and Fox, 1980b). An increase of approximately 6% moisture content was observed when the salt content was increased to 4.5% (QR_3 and QR_4). Table 5 also contains data for the percentage moisture of several commercial Blue cheeses. The percent moisture of commercial samples was in the range of 41 - 46% which is comparable with the values obtained for QR cheese samples. Similar results were reported by Harte (1974) and Dokhani (1978).

Moisture content is important because of the legal standards established for Blue cheese. The composition of Blue cheese specified in the Federal Standards of Identity

(21 CFR, Sec.19.565) is 42 to 46% moisture and not less than 50% fat in the dry matter. According to these standards, end of study results based on moisture content indicated that only cheeses in gas flushed atmosphere packaged cheese ripened at 10°C and control cheeses (unpackaged) ripened at 15°C may present problems that would affect their marketability. The remaining batches are within the legal standards.

Table 5.

Moisture Content of Quick Ripened and Commercial Blue cheeses.

Cheese Sample(a)	% Moisture*
Danish	43.12
Roquefort	41.43
Domestic I	45.69
Domestic II	46.54
QR_1	47.25
QR_2	42.74
QR_3	42.85
QR_4	45.42

(a) Domestic I and II: Blue cheese crumbles, different brand, purchased at the same time.

QR_1 and QR_2 : salted on days 4,5,6 and ripened for two weeks at 10°C and 15°C, respectively and 95% RH

QR_3 and QR_4 : salted on 1st day with 3.5% and 4.5% salt concentration, respectively, and ripened for 3 weeks at 10°C/95% RH.

* Triplicate analysis

Liquid Chromatographic Determination of Methyl Ketones (as 2,4-DNPH Derivatives)

Extraction and Fractionation of Carbonyl Compounds in cheese fat:

Carbonyl-free hexane was used to extract the fat from a homogeneous mixture of cheese and dried celite. The hexane-fat mixture was then applied to a 2,4-DNPH reaction column to convert all of the compounds with carbonyl groups to their 2,4- DNP-hydrazones. The hexane effluent from the 2,4-DNPH reaction column contained all of the original lipids and 2,4-DNP-hydrazone derivatives of carbonyl compounds. The hexane effluent was concentrated and applied to a celite 545-adsorptive magnesia column. The fat was separated from the hydrazones by the addition of a sequence of solvents: hexane, hexane-benzene and benzene. The hydrazones remaining on the column appeared as a deep brown colored band. After elution of the hydrazones using a mixture of chloroform-nitromethane, several blue and grey bands remained adsorbed on the column. These remaining bands were attributed to 2,4- DNP- hydrazones of dicarbonyl compounds (Schwartz et al., 1963). The chloroform-nitromethane eluant containing hydrazones of monocarbonyls and ketoglycerides was reconstituted in hexane and fractionated on a weak alumina column to remove the ketoglycerides from the samples. The monocarbonyl fraction

was evaporated to dryness and dissolved in acetonitrile before injecting an aliquot into the High Performance Liquid Chromatograph (HPLC).

High Performance Liquid Chromatographic (HPLC) Separation of Methyl Ketones

A typical HPLC separation of 2,4-DNP-hydrazones of a standard mixture of C_3 - C_{13} methyl ketones, prepared according to Shriner et al. (1964), is shown in Figure 16. The data represent the HPLC separation conditions of the 2,4-dinitrophenylhydrazone derivatives of 2-propanone (C_3) through 2-tridecanone (C_{13}). Effective resolution of the HPLC peaks was obtained when a reverse-phase analytical column (u-Bondapak, C_{18}) and an eluant of 80 : 20 (v/v) of acetonitrile : water was used (Figure 17). A typical analysis time of 23 min. was required for complete elution of the monocarbonyl derivatives. In Table 6. are shown the HPLC retention times obtained under these conditions. These retention times were established by injecting twice each individual standard ketone derivative. Their respective retention times were then used to identify the individual methyl ketones in Blue cheese samples. Slight variations in retention times of individual ketone derivative in the standard mixture and the sample isolates were observed and are likely due to variation in ambient temperature or purity of samples (Dokhani, 1978).

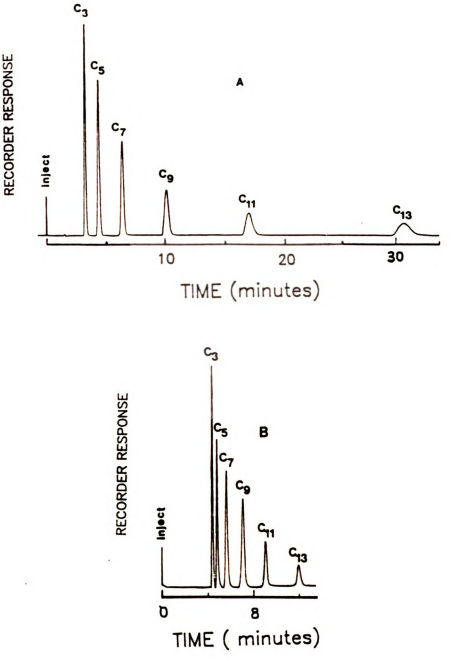


Figure 16. HPLC resolution of 2,4-DNPH derivatives of standard methyl ketones, C_3-C_{13}, at different solvent ratio of acetonitrile : water.
A) 75:25 (V/V) ; and B) 90:10 (V/V)

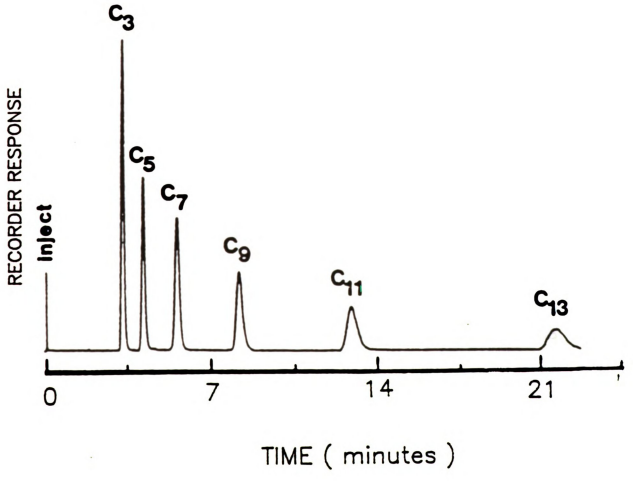


Figure 17. Typical HPLC separation of 2,4-DNPH derivatives of standard methyl ketones, C_3-C_{13}, prepared in solution.
Mobile Phase: Acetonitrile/water
80 : 20 (V/V)

Further HPLC analysis showed the presence of a minor chromatographic peak preceding each major peak of C_8 - C_{13} methyl ketone derivative (Figures 18 and 19). These minor fractions were obtained only whenever a mixture of standard C_8 -C_{13} methyl ketones or the monocarbonyl extracts from Blue cheese samples were passed through the DNPH-reaction column.

Table 6.

The retention times of standard 2,4-DNP-hydrazones of methyl ketones vs. those in Blue cheese samples.

Methyl ketone	Retention time, min.*	
	Standard	Blue cheese
2-Propanone	3.10	3.16
2-Pentanone	4.00	4.05
2-Heptanone	5.55	5.65
2-Nonanone	8.40	8.35
2-Undecanone	13.55	13.70
2-Tridecanone	22.95	22.90

* Average values.

It appears that the derivatization and purification steps involved in methyl ketone analysis were responsible for the formation of these minor compounds.

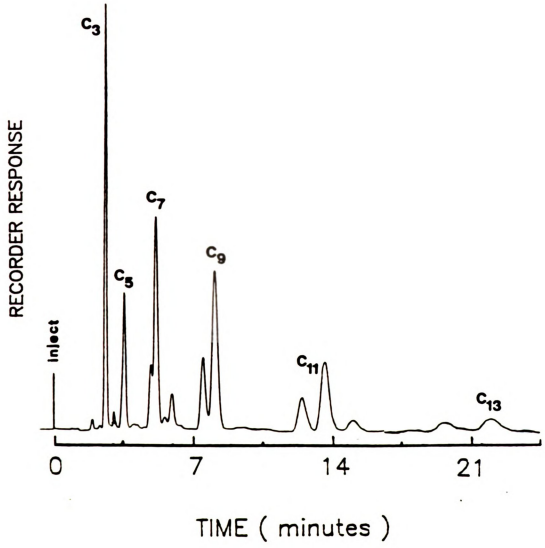


Figure 18. HPLC separation of 2,4-DNPH derivatives of methyl ketones, C_3-C_{13}, present in commercial Blue cheese samples.

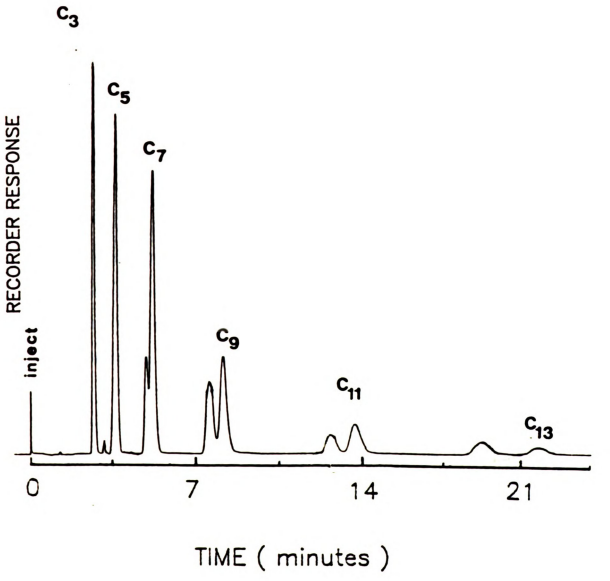


Figure 19. HPLC separation of 2,4-DNPH derivatives of standard methyl ketones, C_3-C_{13}, passed through Celite/Magnesia and Alumina columns.

Mass Spectral Identification of the HPLC minor compounds:

A gas chromatographic-mass spectrometric analysis was performed using a Hewlett-Packard 5985A Gas Chromatograph/ Mass Spectrometer/ Data System (GC/MS/DS) under the following conditions: (GC column: 20 meter x 0.32 mm ID fused silica, J&W DB-54; Temperature program: 60°C (2 min) 10°C/min 300°C (5 min); Injector: Splitless mode at 250°C with a vent pressure delay of 0.75 min.; Ionization energy: 70 eV.; Pressure: 2x 10^{-4} torr; mass range: 45 to 500 amu.

Figure 20 illustrates a typical capillary column GC-MS Total Ion Current (TIC) chromatogram of a mixture of 2,4-DNP-hydrazones of C_5 - C_{13} methyl ketones. Scans # 1034 and # 1008 (retention time 18.28 and 18.78 min , respectively), representing the minor and major components of the C_8-methyl ketone derivative, exhibited similar fragmentation patterns (Figure 21). Similarly, the minor and major components of C7- and C9-hydrazone derivatives were shown to exhibit similar mass fragmentation patterns (Figures 22 and 23). The base peak (100% relative abundance) in the spectra of the minor and major components were at m/z 226, 97, and 69 for 2-pentanone, 2-heptanone and 2-nonanone derivatives, respectively. Additionally, good agreement within the instrumental error, T (less than 10%) was obtained when fragments of ion abundance higher than 10% of their base peaks were compared in both spectra (Tables 7, 8 and 9).

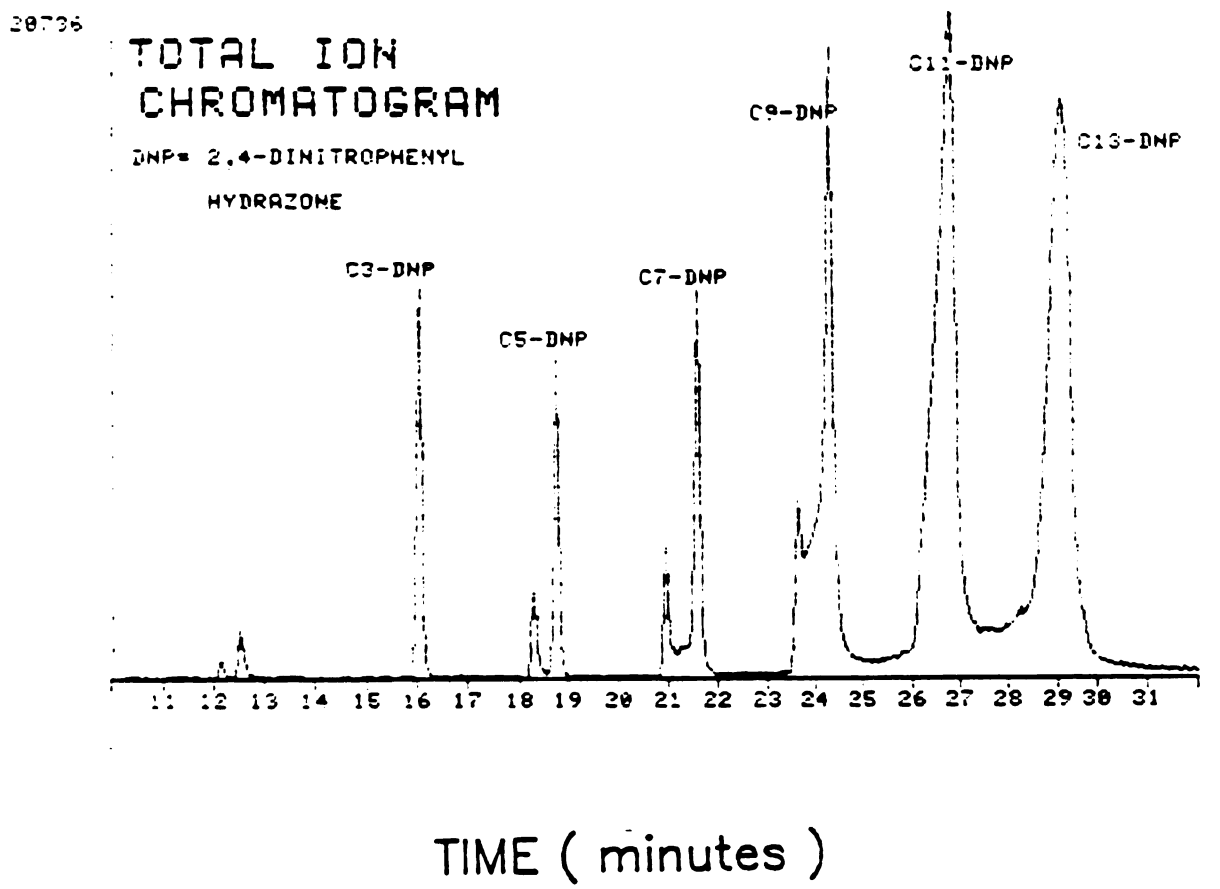


Figure 20. Typical GC/MS total ion current chromatogram of 2,4-DNPH derivatives of standard methyl ketones, C_3–C_{13}.

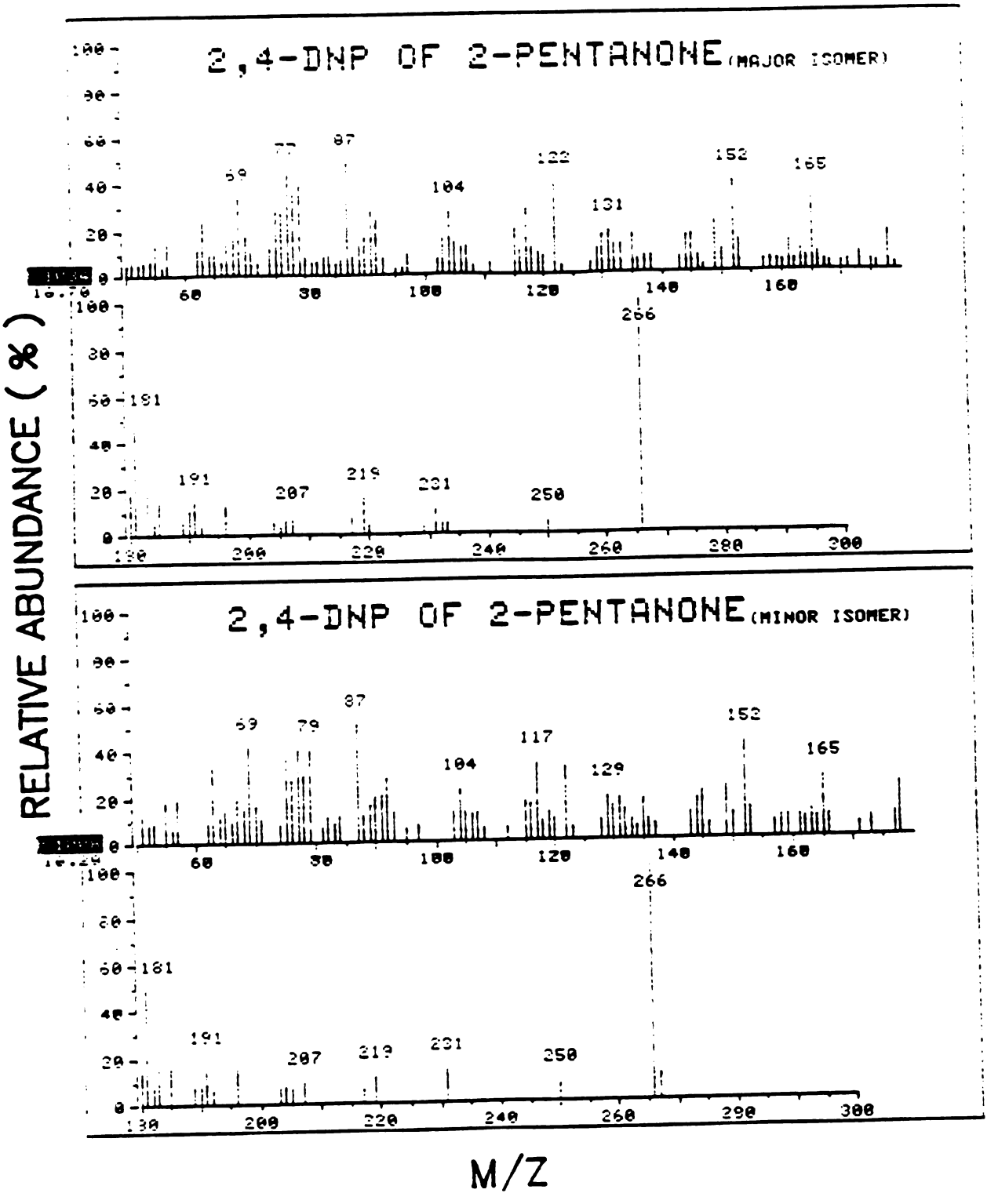


Figure 21. Mass spectrometry patterns of 2,4-DNPH derivatives of standard 2-Pentanone.

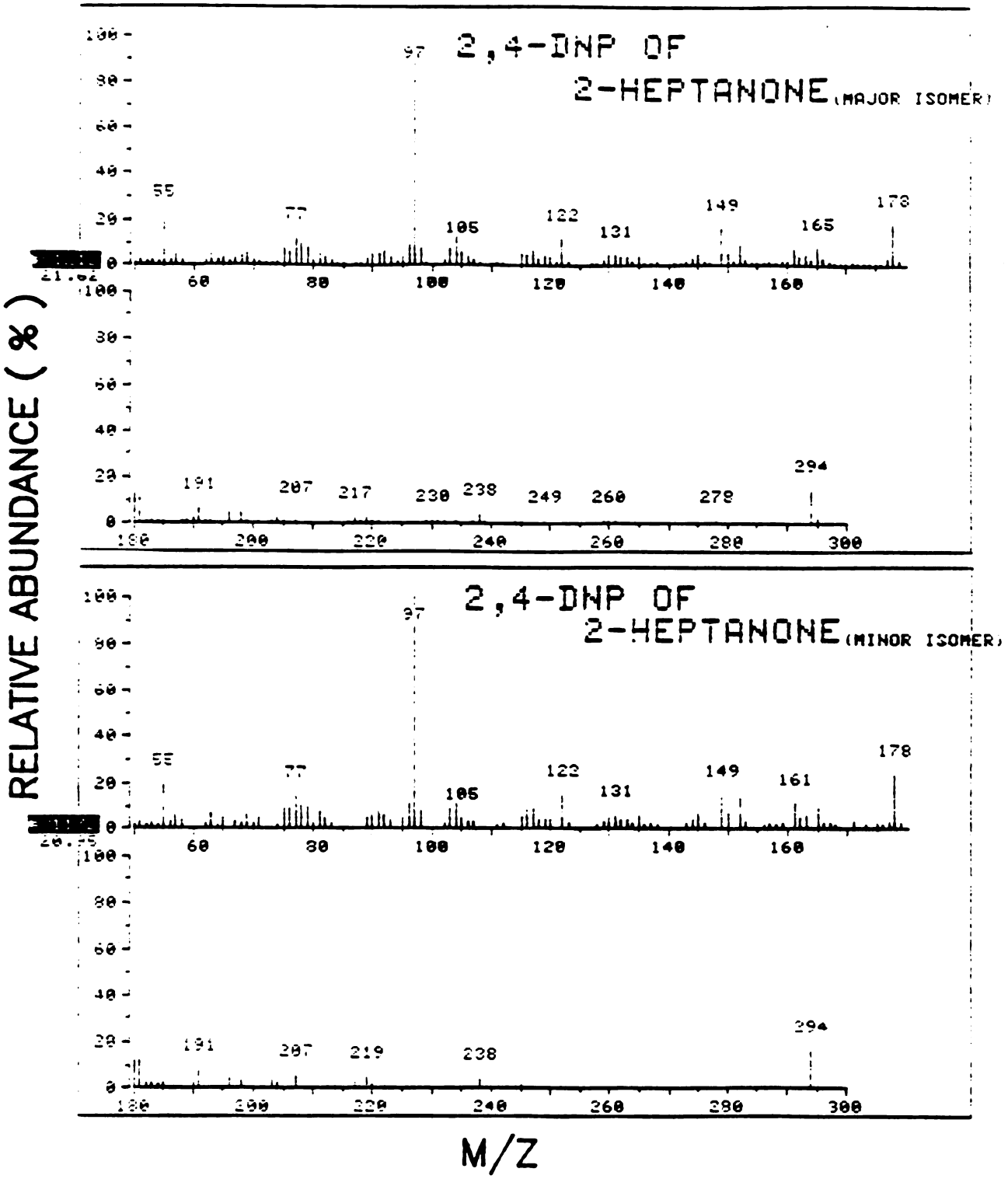


Figure 22. Mass spectrometry patterns of 2,4-DNPH derivatives of standard 2-Heptanone.

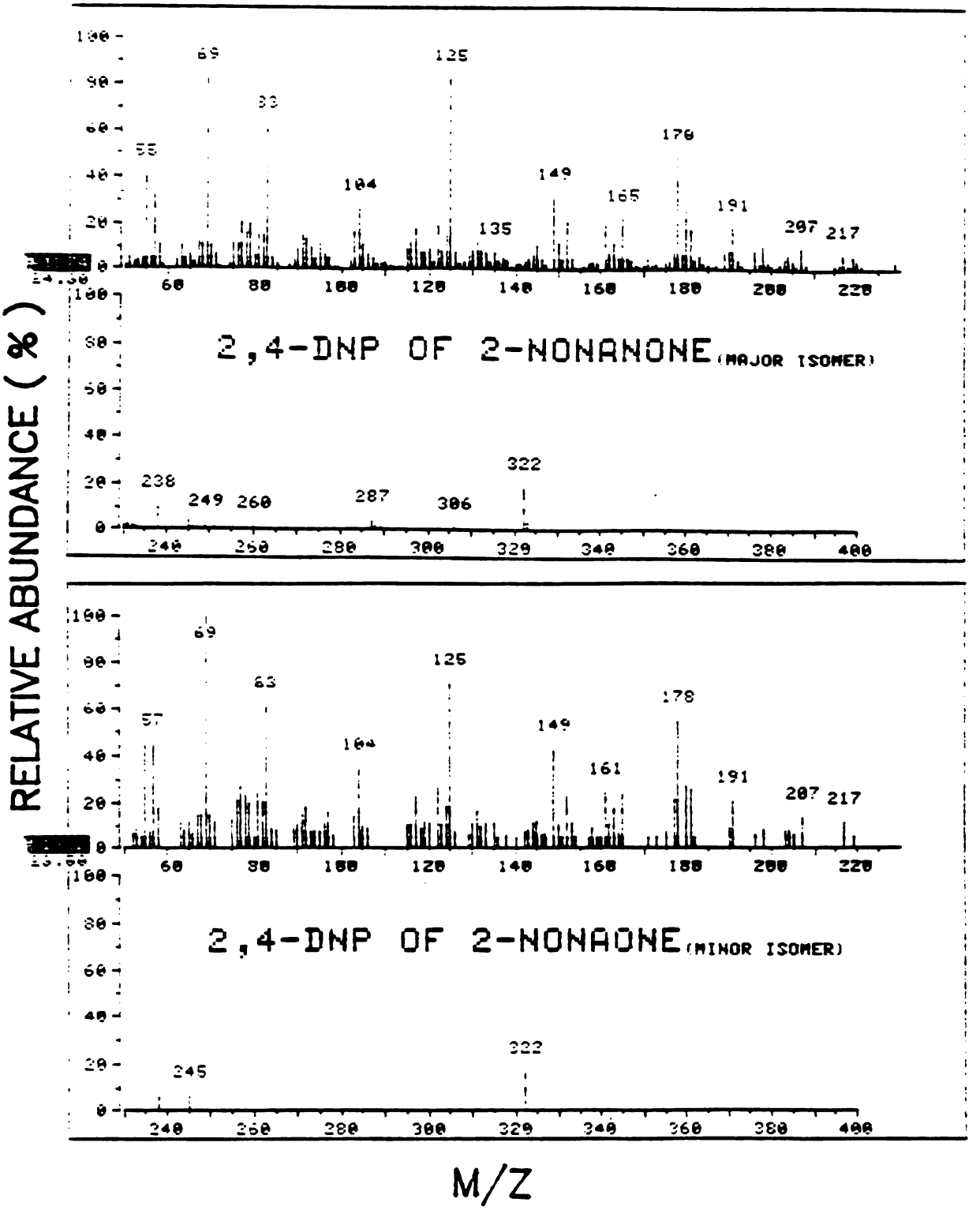


Figure 23. Mass spectrometry patterns of 2,4-DNPH derivatives of standard 2-Nonanone.

The similarity of molecular weight ions and the ion fragmentation patterns of C_8-, C_7- and C_9- methyl ketone derivatives, as shown in their respective spectra (Figures 21, 22 and 23), suggests that these minor HPLC components are geometric isomers, anti- (or trans) or syn- (or cis), of the major components of hydrazone derivatives of C_8 - C_{13} methyl ketones.

Table 7.

Relative abundance comparison of C_8- and C_8* for ion fragments higher than 10% of base peak.

m/z (amu)	relative abundance (%)		
	C_8	C_8*	T▲
266	100.0	100.0	0.0
69	23.2	25.6	2.4
75	25.1	22.3	2.8
77	26.5	23.7	2.8
78	28.8	28.4	0.4
79	27.7	27.4	0.3
87	47.3	40.0	7.3
91	16.5	20.0	3.5
104	18.0	20.5	1.5
117	19.5	25.6	6.1
122	34.1	28.4	5.7
152	34.0	33.0	1.0
165	25.5	25.6	0.1
181	46.4	40.5	5.9

* minor isomer

▲ difference in relative abundance of C_8 and C_8* at different ion fragments.

Table 8.

Relative abundance comparison of C_7^- and C_7^* for ion fragments higher than 10% of base peak.

m/z (amu)	relative abundance (%)		
	C_7	C_7^*	T▲
97	100.0	100.0	0.0
55	21.0	19.2	1.8
77	12.1	14.2	2.1
104	12.9	13.3	0.4
122	12.2	14.8	2.6
149	16.3	14.8	1.5
152	10.0	14.3	4.3
178	18.6	23.3	4.7
180	12.3	11.8	0.5
181	10.2	13.3	3.1
294	13.9	15.7	1.8

* minor isomer
▲ difference in relative abundance of C^7 and C_7^* at different ion fragments.

Table 9.

Relative abundance comparison of C_9^- and C_9^* for ion fragments higher than 10% of base peak.

m/z (amu)	relative abundance (%)		
	C_9	C_9^*	T▲
69	100.0	100.0	0.0
55	42.0	45.1	3.1
57	29.6	35.1	5.5
77	21.8	24.1	2.3
79	18.5	23.5	5.0
83	59.1	59.7	0.6
104	27.6	24.1	3.5
117	20.5	27.2	6.7
122	24.4	22.0	2.4
125	86.3	75.8	10.5
149	31.0	32.5	1.5
152	21.5	20.9	0.6
161	21.5	28.7	7.2
165	25.2	24.6	0.6
178	46.3	52.2	5.9
180	27.4	22.9	4.5
191	18.2	20.0	1.8
322	20.8	22.0	1.2

* minor isomer
▲ Difference in relative abundance of C_9 and C_9^* at different ion fragments.

Quantitation of individual methyl ketones (C_3 - C_{13}):

The calibration curves of 2,4-DNP-derivatives of the standard methyl ketones were prepared by injecting different volumes of known concentrations in acetonitrile into the HPLC. Triplicate analysis were performed and the slopes, correlation coefficients and intercepts were obtained utilizing a programmed calculator (Table 10). The Linearity of the standard curves were excellent and were used for the quantitation of methyl ketones in samples.

Table 10.

Values of slopes, correlation coefficients and intercepts of calibration curves of 2,4-DNPH-derivatives of C_3-C_{13} methyl ketones.

Methyl ketone	Slope*	Correlation Coefficient	Intercept
2-Propanone	250.02	0.9987	8.5131
2-Pentanone	154.73	0.9993	4.5011
2-Heptanone	88.34	0.9994	2.1425
2-Nonanone	59.10	0.9995	1.8513
2-Undecanone	30.50	0.9997	0.6834
2-Tridecanone	13.91	0.9998	0.2034

* Average of duplicates.

Recovery study of C_3-C_{13} methyl ketones extractions:

For more reliable quantitation of methyl ketones, it was necessary to determine the efficiency of the extraction and chromatographic procedures. An accurately weighed mixture

of standard C_3-C_{13} methyl ketones (99.5% purity) were spiked into a 250 g cheese sample and subjected to the extraction and chromatographic procedure (Experimental section). An identical blank cheese sample was subjected to the same procedures and aliquots of extracts were injected into the HPLC. The calculated concentrations were then compared to those of untreated derivatives of standard C_3-C_{13} methyl ketones. The percent recovery of the combined minor and major isomers of each standard methyl ketone derivative (Table 11), as determined by duplicate analyses, was calculated and used in estimating the concentration of ketones in the cheese samples.

Table 11.

Percent recovery of standard C_3 - C_{13} methyl ketones.

Methyl Ketone	Average Percent Recovery*
2-Propanone	81.35 ± 0.86
2-Pentanone	83.73 ± 0.48
2-Heptanone	93.38 ± 1.23
2-Nonanone	94.22 ± 0.95
2-Undecanone	97.50 ± 0.72
2-Tridecanone	98.72 ± 0.83

* The average recovery of triplicate analyses. This average was used in quantitating the methyl ketones in cheese samples.

The percent recovery was lower for lower molecular weight methyl ketone derivatives, this was also reported by Blakely (1970) and Dokhani (1978). The molecular weights of methyl ketones and their 2,4-DNP-hydrazones are presented in Appendix A. In using the external standard method of quantitation, the HPLC response of a component was directly compared to its respective calibration curve and accounted for different detector responses of sample components by multiplying peak areas by a response factor.

Methyl ketones in commercial imported and domestic Blue cheese:

The concentration of individual methyl ketones in Blue cheese samples were calculated as micromoles (uM) ketone per 10 g fat in cheese (Blakely,1970), Albert (1974) and Dokhani (1978). The methyl ketone concentrations in several commercial and domestic Blue cheese samples are presented in Table 12. The major methyl ketones in the samples were 2-heptanone and 2-nonanone. However, high concentration of 2-propanone and 2-pentanone were also observed in these samples. The 2-undecanone was present in the lowest concentration while 2-tridecanone was not detected in most of the Blue cheese samples. Variation in methyl ketone concentrations within the same or different brands of Blue cheese samples was also found by Blakely (1970), Albert (1974) and Dokhani (1978).

Table 12.

Methyl ketones concentration of Imported and domestic commercial Blue cheese

Blue cheese sample*	uM methyl ketones /10 g fat						
	C_3	C_5	C_7	C_9	C_{11}	C_{13}	Total
Danish I	3.82	8.89	13.5	12.13	2.9	1.21	42.45
Danish II	9.29	13.25	15.25	11.45	3.24	0.80	53.28
Roquefort	1.92	0.95	8.35	7.39	0.92	-	19.53
Domestic^{a1}	9.33	15.46	21.73	9.13	2.85	-	58.50
Domestic^{a2}	12.46	17.66	25.84	10.29	1.81	-	68.06

* Danish I and II: purchased at different local markets. Domestic^{a1} and ^{a2} : different brands.

The analysis of methyl ketones in Roquefort cheese (Figure 24B and Table 12) revealed that C_5, C_7 and C_9 to be the dominant ketones. Other ketones were found in concentrations below 2.0 uM per 10 g fat and C_{13} was not detected. The above findings confirmed the results obtained by Starkel (1924) and Dokhani (1978).

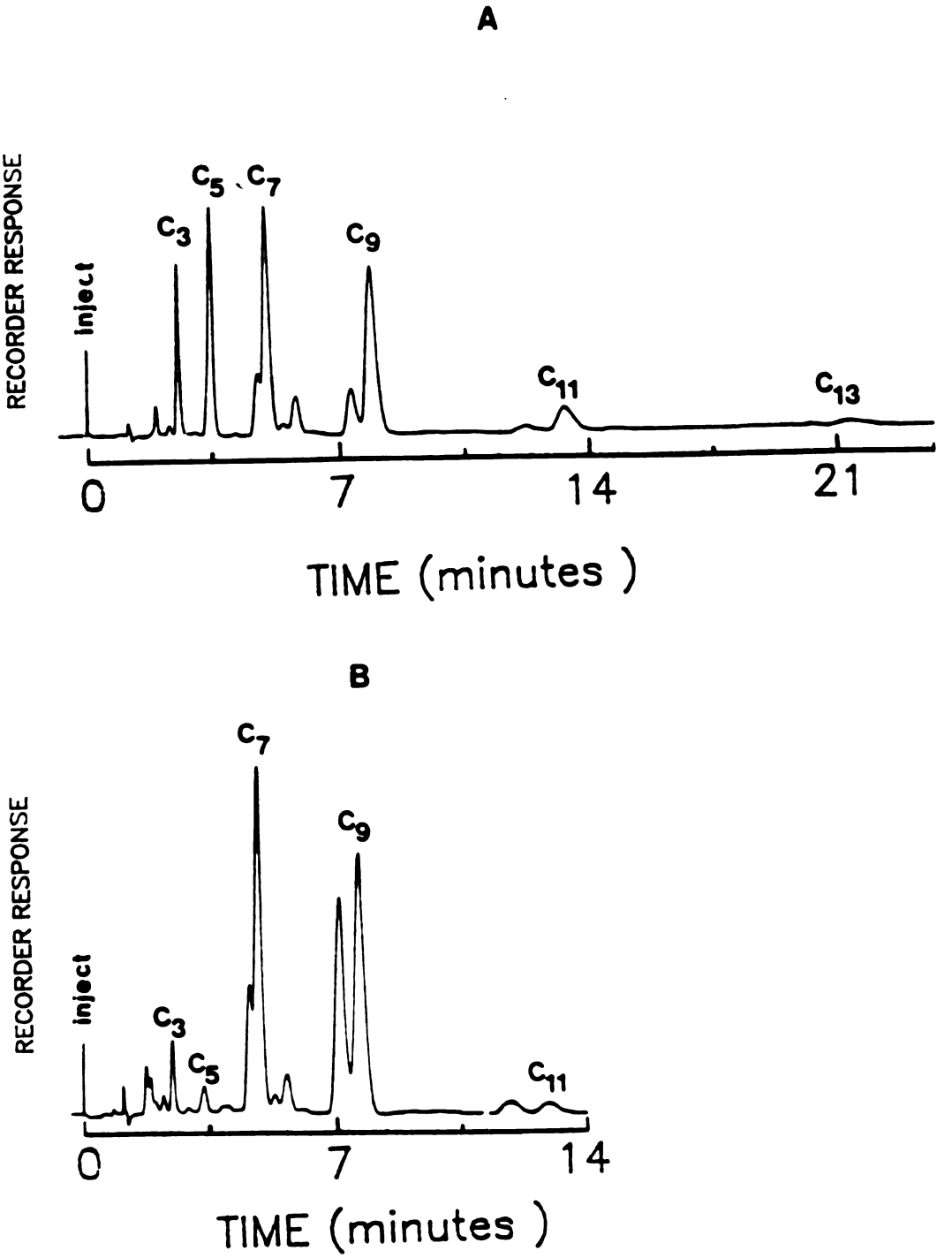


Figure 24. Typical HPLC separation of 2,4-DNPH derivatives of methyl ketones in commercial: A) Danish ; B) French Roquefort

Methyl ketones in Quick Ripened (QR) Blue cheese in controlled atmosphere packaging:

The development of methyl ketones in batches of Blue cheese ripened in modified and gas flushed atmosphere packaging are presented in Table 13. During the two weeks in the curing room, various concentrations of ketones resulted. The ripening temperature and the packaging procedures were responsible for this variation. The gas flushed atmosphere cheeses exhibited the highest concentration of ketones. This can be attributed to the increased mold growth due to the rich oxygen atmosphere over the packaged cheese (Golding, 1940 II). The cheeses in modified atmosphere packaging had the lowest concentration of ketones as a result of restricted mold growth. The C_7 methyl ketone was present in highest amount in all cheeses. Generally, C_5 and C_7 methyl ketones together accounted for more than 50% of the total methyl ketones. The predominant ketones reported by Blakely (1970), Albert (1974) and Dokhani (1978) under different ripening conditions were C_7 and C_9 methyl ketones. The C_{11} methyl ketone was the lowest concentration while C_{13} methyl ketone was not detected in most of the batches. An increase of 25 - 50% in the total ketone concentrations was obtained as a result of employing a higher ripening temperature (15°C). This was likely due to increased mold growth and enzymatic activity.

Table 13.

Methyl ketones concentration of Blue cheese quick ripened in modified and gas flushed atmosphere packaging for 2 weeks at 10°c and 15°C/ 95% RH.

Methyl ketone	uM methyl ketones/ 10 g fat*					
	Control		Modified atmosphere		Gas flush. atmosphere	
	10°C	15°C	10°C	15°C	10°C	15°C
2-Propanone	0.35	0.80	0.25	0.60	0.35	0.94
2-Pentanone	1.60	2.10	0.90	1.65	3.70	4.44
2-Heptanone	1.50	2.85	1.95	2.40	4.92	5.15
2-Nonanone	0.90	1.20	0.65	1.24	2.19	3.11
2-Undecanone	0.77	0.95	0.50	0.61	0.61	0.89
2-Tridecanone	-	-	-	-	-	-
Total	5.12	7.90	4.25	6.50	11.77	14.53

* Duplicate analysis.

In Table 14 , are presented the methyl ketones concentration of the ripened cheese at the end of two weeks storage at 5°C. A 3-4 fold increase in the total methyl ketone concentrations was observed for the control (unpackaged) and the modified atmosphere packaged cheeses. The increase in ketones production coincided with an increase in the mold growth. The C_9 methyl ketone concentration increased approximately five fold. This enhanced methyl ketone production was attributed to the progressive increase in the activity of beta-ketoacyl

decarboxylase as the spores germinated (Hawke, 1966). After three weeks, there was a slight decrease in the total methyl ketone concentrations for cheeses in gas flushed atmosphere. This may be explained by the reduction of ketones to secondary alcohols (Kinsella and Hwang, 1977) and the anaerobic fermentation may have occurred as a result of the

Table 14.

Methyl ketones concentration of Blue cheese quick ripened in modified and gas flushed atmosphere packaging for 2 weeks at 10°C and 15°C plus two weeks stored at 5°C/ 95% RH.

Methyl ketone	uM methyl ketones/ 10 g fat					
	Control		Modified atmosphere		Gas flush. atmosphere	
	10°C	15°C	10°C	15°C	10°C	15°C
2-Propanone	2.85	3.11	0.73	0.95	0.93	1.13
2-Pentanone	4.61	6.10	5.15	6.13	4.20	4.64
2-Heptanone	7.35	8.25	5.35	6.98	3.83	3.84
2-Nonanone	5.10	6.35	4.36	5.13	1.10	2.95
2-Undecanone	1.15	1.79	0.92	1.40	0.65	0.91
2-Tridecanone	0.40	0.50	-	-	-	-
Total	21.46	26.10	16.51	18.45	10.71	13.47

* Duplicate analysis.

extremely low oxygen level in the headspace. The development of an off-flavor and the loss of the blue-veined quality of cheeses in gas flushed packages were also observed. This phenomenon was noted by Kosikowski (1977) when the blue cheese was vacuum packaged and stored in a cold room.

It appeared that the methyl ketones development in QR Blue cheese samples exhibited a pattern similar to that observed in commercial Blue cheese ripened over several months. However, QR Blue cheese samples exhibited lower methyl ketones concentration than the commercial Blue cheeses.

Sensory Evaluation of QR Blue Cheese in Controlled Atmosphere Packaging:

The sensory analyses of QR Blue cheese ripened at 10°C and 15°C for two weeks are presented in Table 15. These cheeses were evaluated for their flavor, texture and color (Appendix B) and a total score of 30 was considered acceptable.

The statistical analysis of sensory evaluation (Appendix C) revealed significant difference ($P < 0.05$) in the total score between QR control, modified and gas flushed atmosphere packages. However, no significant difference ($P < 0.05$) was observed between ripening temperature. A significant increase ($P < 0.01$) in total score was observed as cheeses ripened in modified atmosphere packaging were

stored at 5°C for additional two weeks. The total scores of cheeses ripened in gas flushed atmosphere packaging decreased significantly ($P < 0.01$) when stored at 5°C for additional two weeks.

The control cheeses ripened at 15°C were criticized for weakness of texture and slightly gummy. Color was well developed due to good mold growth and flavor was slightly bitter, presumably due to the extensive protein degradation. The control cheeses at 10°C had a well balanced, moderate flavor without trace of off-flavor. The texture was slightly weak and the mold growth was acceptable but not optimal. The modified atmosphere cheeses ripened at 10°C were criticized for lack of mold growth and mildness of flavor while the cheeses ripened at 15°C had good mold growth and a slightly mild flavor. The gas flushed atmosphere cheeses ripened at 10°C and 15°C were graded superior to all other cheeses. The flavor was excellent, texture was slightly firm and mold growth was very apparent. This was likely due to the rich oxygen atmosphere in the headspace during the first week of ripening. The overall score for control cheeses was highest for the lower ripening temperature (10°C) while cheeses ripened at 15°C in modified and gas flushed atmosphere were graded higher than these at 10°C. All the cheeses ripened at 10°C and 15°C for two weeks were acceptable.

Table 15.

Subjective analyses[a] of QR Blue cheese ripened at 10°C and 15°C for two weeks.

Cheese	Flavor (20)		Texture (10)		Color (15)		Total[b] (45)	
	10°C	15°C	10°C	15°C	10°C	15°C	10°C	15°C
Commercial[c]	18	18	9	9	14	14	41	41
QR Control	14	13	8	7	12	13	34[b]	33[c]
Modifd.Atms.	14	14	8	9	10	12	32[c]	35[b]
Gas Flush.	15	17	9	9	13	13	37[a]	39[a]

[a] Standard error is presented in Table B.2.
[b] Means within each column not followed by the same letter are significantly different ($P < 0.05$).
[c] Aged at least six months.

In Table 16 are presented the subjective analyses of QR Blue cheeses ripened at 10°C and 15°C for two weeks and then stored at 5°C for an additional two weeks. After two weeks storage at 5°C, the control cheeses ripened at 15°C were criticized for excessive mold growth, which resulted in musty flavor, gummy texture, and dehydrated appearance while the flavor of cheeses ripened at 10°C improved slightly. The cheeses in modified atmosphere were graded superior in all quality categories. These cheeses were slightly firm, excellent flavor and with optimum mold growth. The cheeses in gas flushed packaging suffered major problems when an extended period of storage was applied.

The blue pigmentation disappeared, presumably due to the vacuum created inside the pouches (Kosikowski, 1977) and/or the low oxygen level. The condensation increased inside the pouches resulting in wetting of the cheese and an atypical flavor developed which could be the result of an anaerobic fermentation (Karel, 1974).

Table 16.

Subjective analyses[a] of QR Blue cheese ripened at 10°C and 15°C for 2 weeks and then stored at 5°C for an additional 2 weeks.

Cheese	Flavor (20)		Texture (10)		Color (15)		Total[b] (45)	
	10°C	15°C	10°C	15°C	10°C	15°C	10°C	15°C
Commercial[c]	19	19	9	9	15	15	43	43
QR Control	16	13	7	6	13	14	36[e]	33[e]
Modifd.Atms.	17	16	9	9	13	13	39[d]	38[d]
Gas Flush.	12	12	8	7	6	7	26[f]	28[f]

[a] Standard error is presented in Table B.2.
[b] Means within each column not followed by the same letter are significantly different ($P < 0.05$).
[c] Aged at least six months.

These changes were detrimental to the flavor, texture and appearance of the cheese. The additional two weeks of storage at lower temperature (5°C) improved the quality of cheeses in modified atmosphere packaging but had an adverse effect on the quality of cheeses in gas flushed atmosphere.

Visual Observations:

One of the main objectives in the present study was to reduce or eliminate the risk of microbial contamination during ripening and storage. The QR Blue cheeses were visually inspected daily for the presence of undesirable contamination and excessive slime formation. The visual observations indicated that the surface of control cheeses developed a slight slime formation at 10°C and 15°C, while slime formation for cheeses ripened in modified and gas flushed atmosphere packaging did not exhibit any apparent slime on the surface. Discoloration, which may result from spoilage organisms was absent in all cheeses. Factors such as early salt application, reduced multiple mixing of the cheese and the protective properties of the package materials may have contributed to the elimination of any apparent microbial contamination.

Physical Methods of Preservation

In QR Blue cheese, excessive mold growth can occur which results in the development of a musty and moldy flavor. Methods of preservation such as ionizing radiation and microwave heating in package were applied to cheeses after ripening for two weeks in LDPE pouches. The objective of these treatments was to retard the excessive mold growth and hence control the accelerated ripening of Blue cheese curd. Following these treatments, the cheeses were stored at 5°C for one week over 95% RH and then evaluated.

A. Ionizing Radiation:

Generally, dairy products may develop objectionable changes in flavor, odor and color when irradiated. These changes depend on several factors such as the product being irradiated, the moisture content, the amount of irradiation (dose), the irradiation temperature and the storage conditions (Urbain, 1986).

The subjective analyses of Blue cheeses irradiated with various doses and stored at 5°C for one week are presented in Table 17. The cheeses irradiated with 0.5 KGy did not develop any objectionable flavor or odor and the color was excellent (described as whitish background and pronounced bluish color). An obvious reduction in mold pigmentation

was observed in cheeses irradiated with 1.0 KGy and a slight change in flavor, though not objectionable, was noted. A dose of 1.5 - 2.0 KGy was sufficient to reduce mold growth (Penicillium spp.) in apples by 80% (Sommer and Fortlage, 1966).

Table 17.

Subjective Analyses* of Blue cheese irradiated and stored at 5°C/ 95% RH for one week.

Dose (KGy)	Flavor (20)	Texture (10)	Color (15)	Total (45)
Control (0)	14	9	14	37
0.5	14	8	13	35
1.0	13	8	11	32
2.0	9	5	9	23
2.5	8	5	8	21

* Done by the investigators.

Significant changes in quality occurred in cheeses irradiated with doses of 2.0 KGy and higher. These cheeses exhibited the typical "irradiated" flavor which is characterized by a bitter and oxidative flavor. Such flavor was attributed to the formation of radiolytic products such as acetaldehyde, dimethyl sulfide, CO_2, aliphatic hydrocarbons, acids and alcohols which have been previously identified in irradiated dairy products (Urbain, 1986). The texture was grainy with a pronounced fading of color.

Cheeses irradiated with 2.0 KGy and higher were not acceptable. In Table 18 are shown the methyl ketone concentrations of irradiated Blue cheese. A marked increase in C_3 methyl ketone concentration (50 - 80%) was observed in cheeses irradiated with 0.5 and 1.0 KGy while a slight decrease was noted for the other methyl ketones. Cheeses irradiated with 2.5 KGy showed significant reduction in concentrations of most methyl ketones though C_3 methyl ketone increased significantly. The decrease in methyl ketone concentrations was likely due to the cleavage which occurred preferentially at bonds in the vicinity of the carbonyl group (Urbain, 1986). The data suggest that irradiation dosage of 1.0 KGy was the optimum, under the experimental conditions, to control mold growth with minimal change in quality of cheese.

Table 18.

Methyl ketones concentration of QR Blue cheese irradiated and stored at 5°C for one week.

Dose (KGy)	uM methyl ketone/ 10 g fat						
	C_3	C_5	C_7	C_9	C_{11}	C_{13}	Total
Control (0)	2.15	5.20	7.95	5.39	1.20	-	21.89
0.5	3.24	5.14	6.80	5.13	0.92	-	21.23
1.0	3.86	4.54	6.14	4.83	0.44	-	19.81
2.5	4.23	1.15	2.45	1.71	0.20	-	9.74

B. Microwave Heating:

The subjective analyses of Blue cheeses subjected to microwave heating "in-Package" with subsequent storage at 5^{o}_{c} for one week are presented in Table 19. The most obvious effect of heating above 39.5°C was clumping and matting of the cheese.

Table 19.

Subjective analyses of Blue Cheese "In-Package", microwave heating with storage at 5°C for one week

Temperature (°C)	Time (sec)	Flavor (20)	Texture (10)	Color (15)	Total (45)
Control (Untreated)		14	9	14	37
29.5	5	14	9	13	36
39.5	10	13	8	11	32
53.0	15	12	5	9	26
71.5	20	10	4	7	21
84.5	25	8	4	6	18

A significant reduction in mold growth was observed for cheeses heated to 53.0°C and above. However, above 53°C the cheese began to liquify and thus these temperatures were detrimental to the flavor and texture of the cheese. There was no change in quality and appearance when cheeses were heated to 29.5°C. An obvious reduction in mold growth was noted for cheeses heated to 39.5°C but the flavor and

texture were slightly affected. The total score indicated that all cheeses heated to 53.0°C and above were not acceptable.

The effect of microwave heating upon the package integrity was also noted. The pouches began to expand, with stretching and thinning of the material, when subjected to 39.5°C and above. Package integrity is critical to product stability. It is essential that the package protects the product from possible contamination during and after the heating process (Tochman, 1985).

The data suggest that a temperature of 39.5°C was optimum in reducing mold pigmentation with only slight effect on the flavor and texture of the cheese.

SUMMARY AND CONCLUSIONS

Blue cheese curd quick ripened in modified and gas flushed atmosphere packaging resulted in a well-flavored Blue cheese. The product is suitable for direct use in salad dressings and dips because of the small, well-veined loose curd particles. This would improve color consistency, texture and overall appearance. Overhead costs would be greatly reduced due to less facilities and warehousing space needed during the manufacturing and ripening of Blue cheese curd. The amount of physical labor required during ripening would be substantially reduced because of the short ripening time and less labor required for maintaining the cheese. The risk of microbial contamination prior to packaging was eliminated and the excessive mold growth reduced due to early salt application, and selecting and maintaining the optimum atmosphere over packaged curd.

To select package materials with optimum barrier properties, the O_2/CO_2 requirements of the Blue cheese curd was investigated. The amount of carbon dioxide produced (respiration rate) and the magnitude of its increase were directly related to the temperature employed in ripening. The CO_2 production ranged from 52.45 - 130.70 cc/kg/hr

depending on the ripening temperature. The amount of oxygen consumed was slightly higher than the amount of CO_2 produced. Temperature of ripening also had a marked effect on cheese quality. Subjective analyses found that cheeses ripened at 20°C were heavily criticized in all quality departments, while cheeses ripened at 5°C lacked mold growth and flavor. Cheeses ripened at 10°C and 15°C were selected for their higher quality.

A correlation was shown to exist between water-soluble nitrogen and pH values. As the water-soluble nitrogen increased, there was a corresponding increase in pH values. The highest pH value attained was approximately 6.03 - 6.98 and stabilized or in some cases decreased to approximately 5.46 - 6.49 by the end of ripening. Approximately 32 - 43% of the total protein was present in the soluble form after ripening. This level was also attained when an extended ripening period was used. The wide variation in pH and water-soluble nitrogen values were attributed to the ripening temperatures employed and the packaging procedures used.

The gas headspace analyses of modified and gas flushed atmosphere packaging revealed that the O_2/CO_2 requirements of P.roqueforti are functions of both concentration and temperature and that a mutually dependent relationship exists between respiration and permeation within the Blue

cheese packaging system.

The moisture content of Blue cheeses prior to packaging was 50.34% and decreased to 41.21 - 46.65% depending on the temperatures employed, the salt content, and the water vapor transmission of the package materials.

A rapid technique, utilizing a High Performance Liquid Chromatography (HPLC) system, was used to separate, identify and quantitate C_3 to C_{13} methyl ketones in QR Blue cheese and commercial Blue cheeses. The 2,4-dinitrophenylhydrazone (DNPH) derivatives of standard methyl ketones were prepared and compared with their corresponding derivatives isolated from Blue cheese. Under the chromatographic conditions used in this study, the DNPH derivatives of C_5 - C_{13} methyl ketones exhibited several related "minor components". These minor components were formed during the derivatization and purification steps. The mass spectral analysis of C_5-, C_7-, and C_9- methyl ketone derivatives and their corresponding minor components indicated their possible geometric isomerization during the derivatization step. Methyl ketone development in QR Blue cheese samples exhibited a pattern similar to that observed in commercial Blue cheese ripened over several months. The predominant methyl ketone in QR Blue cheese was C_7 with considerable amount of C_5 and C_9 found. The C_{11} methyl ketone was present in the lowest concentration while C_{13} methyl ketone was not detected in

most of the batches. An increase of 3 - 4 fold in the total methyl ketone concentrations was observed when control (unpackaged) and modified atmosphere packaged cheeses were stored at 5°C for an additional two weeks following ripening. Generally, a large variation in the concentration of methyl ketones was observed among the cheese samples due to the temperatures and treatments employed.

The subjective analyses of QR Blue cheese revealed that:

a. Cheeses ripened in gas flushed atmosphere were superior to other QR cheeses after two weeks ripening. However, these cheeses suffered major problems when the cheese was stored for an extended period at 5°C.

b. Cheeses ripened in modified atmosphere were graded acceptable in all quality categories and the quality improved substantially when the cheese was stored for an extended period at 5°C.

c. Generally, cheeses ripened at lower temperatures such as 10°C and stored for two weeks at 5°C were of superior flavor.

An obvious reduction in mold growth or pigmentation was observed in cheeses irradiated with 1.0 KGy and a slight decrease in methyl ketone concentrations was detected. A slight change in flavor, though not objectionable, was observed.

The subjective analyses of QR Blue cheese subjected to microwave heating "In-Package" revealed that a temperature of 39.5°c was the optimum in reducing mold pigmentation with a slight effect on the flavor and texture of the cheese.

The research reported herein demonstrated the feasibility of quick ripening Blue cheese curd by controlled atmosphere packaging. A significant reduction in the total cost of cheese manufacture and storage could result provided that a well-balanced flavor could be maintained throughout the distribution.

Further research is needed in modifying, improving and refining the accelerated ripening of Blue cheese in controlled atmosphere packaging. In conjunction with this process, several possibilities can be investigated such as:

1. The addition of modified lactic acid bacteria to cheese milk and/or bacterial concentrates to the cheese curd prior to packaging.
2. The use of lactose to stimulate the growth of normal culture and probably encourage the growth of P.roqueforti.
3. The addition of microbial enzymes, particularly fungal lipase, and/or free fatty acids to the cheese curd prior to packaging.

Further research is also needed for more practical methods to control the accelerated ripening without

impairment of flavor balance. The use of refrigerated and/or freezing temperatures of storage would be a possibility

Several aspects of the biochemistry of P.roqueforti and strain variations remain to be described in order to improve the production and flavor development of Blue cheese and to determine their nutritional value and safety.

APPENDICES

APPENDIX A

Table A.--The molecular weight of methyl ketones[a] or their 2,4-Dinitrophenylhydrazone derivatives.

Methyl ketone chain length	M.W. of non-derivatized	M.W. of derivatized[b]
C_3	58.8	238.94
C_5	86.1	266.24
C_7	114.2	294.34
C_9	142.2	322.34
C_{11}	170.3	350.44
C_{13}	198.4	378.54

(a) Values taken from Handbook of Chemistry and Physics, 1975-1976.

(b) The molecular weight of derivatives were calculated as following:

M.W. derivative =

= (M.W. methyl ketone + M.W. 2,4-dinitrophenylhydrazine)- M.W. H_2O

where

M.W. 2,4-dinitrophenylhydrazine = 198.14

M.W. H_2O = 18.0

APPENDIX B

Q.R. BLUE CHEESE SCORE SHEET

Name: _____

Date: _____

Sample #: _____

A. Color-Mold Growth Appearance (15)

0 ——— 15

Please describe the color:

___ No mold growth
___ Lacking mold growth
___ Slight mold growth
___ Excessive mold growth
___ Excellent (explain)

B. Texture (10)

0 ——— 10

Please describe the texture:

___ Very weak, gummy
___ Weak, mealy, slightly gummy
___ Slightly weak, slightly mealy
___ Slightly firm, excellent
___ Other (explain)

C. Flavor (20)

0 ——— 20

Please describe the flavor:

___ Atypical, moldy, sour, unclean, very bitter
___ Bitter, soapy
___ Mild, slightly bitter, slightly soapy
___ Slightly mild, excellent (well blended)
___ Other (explain)

APPENDIX C

Table B.1

Analysis of variance for sensory evaluation

Source	Degree of Freedom	Sum of Squares	Mean Square	F-value	Probability*
Replicates (A)	1	9.05	9.054	5.89	*
Panelists (B)	6	0.04	0.006	0.00	NS
AxB	6	26.99	4.498	2.93	**
Pkg.treatments (C)	2	348.68	174.339	76.50	*
AxC	2	6.04	3.018	1.96	NS
BxC	12	9.57	0.798	0.52	NS
Temperatures (D)	1	5.01	5.006	3.26	NS
AxD	1	0.01	0.006	0.00	NS
BxD	6	0.20	0.034	0.02	NS
CxD	2	123.01	61.506	40.04	**
Storage times (F)	1	115.01	115.006	74.86	**
AxF	1	1.34	1.339	0.87	NS
BxF	6	1.04	0.173	0.11	NS
CxF	2	1932.01	966.006	628.84	**
DxF	1	43.01	43.006	28.00	**
CxDxF	2	27.01	13.506	8.79	**
Residual (E)	115	176.66	1.536		

* NS = not significant
* = significant at a = 0.05
** = significant at a = 0.01

Table B.2

Standard error (ST_E) for the main factors in the statistical model

Factors	Replicates	Panelists	Package treatments	Temperatures	Storage times
Storage times	0.19	0.36	0.23	0.19	0.14
Temperatures	0.19	0.36	0.23	0.14	
Package treatments	0.23	0.44	0.17		
Panelists	0.36	0.25			
Replicates	0.14				

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