This is to certify that the

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"IN-PACKAGE RIPENING OF LOOSE

BLUE CHEESE CURDS

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"IN-PACKAGE" RIPENING OF LOOSE BLUE CHEESE CURDS

By

Crystal Aque Morrison

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ABSTRACT

"IN-PACKAGE" RIPENING OF LOOSE BLUE CHEESE CURDS

By

Crystal Aque Morrison

In this study, "in-package" ripening of Blue cheese was evaluated. Prior to packaging, cheese curds were inoculated with <u>Penicillium</u> <u>roqueforti</u> then packaged in these three flexible materials: Low-density polyethylene (LDPE), Ethylene-vinyl acetate (EVA) and Laminated aluminum foil pouches (LAF).

Cheese curds were either packaged under ambient conditions or flushed with a 30% oxygen enriched atmosphere.

All packages were stored in a temperature controlled chamber (approximately 52F) and 55-60% relative humidity for a minimum of 10 days and a maximum of 18 days.

The initial whey in the curds, permeability of the packaging materials, and storage conditions contributed to a relative humidity of 95% inside of the pouches.

Curds ripened in 7-10 days, as compared to 90 days to 1 year using conventional processes. Variables included: barrier materials, salt, mold and packaging atmosphere.

The ripening of the cheese was monitored by measuring pH,

gas headspace, moisture content, spoilage organisms, and sensory evaluation. Packages of cheese from each of the three barrier materials were evaluated daily. Sensory evaluation revealed, cheese packaged under accelerated conditions in LDPE ripened most favorably. Cheese curds packed in EVA and LAF pouches were least favorable.

During ripening, pouches of cheese containing 0% salt showed signs of microbial growth slightly sooner than cheese with 1 or 2% salt. Two different concentrations (1/2 and 1%) of P. roqueforti spores were added to the non-ripened curds. There was no significant difference with the rate curds ripened due to concentration of mold spores. Overall, packages of curds flushed with an oxygen enriched atmosphere ripened slightly sooner than curds packaged under ambient conditions. Visual changes in the curds were noted throughout the study. Cheese curds were considered palatable up to 10 days of "in-package" ripening.

In loving memory of my grandmother,
Marguerite Ann Morrison.
My husband, Lydell Woods.
My parents, Thomas and Priscilla Foster.
My entire family and special friends, Nadia A., Laura B.,
Linda B., Donna E., Nancy H., Sara M., and Mary M.

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INTRODUCTION

The conventional method of manufacturing loose curd Blue cheese utilizes large amounts of preparation and storage space. There is also the risk of microbial contamination to the cheese prior to packaging. Hence, the need for a more efficient method to manufacture loose curd Blue cheese.

The purpose of this study was to determine the feasibility of "in-package" ripening of Blue cheese using controlled atmosphere packaging.

Barrier materials with different gas transmission rates and headspace environments influenced oxygen levels, and ripening of the cheese curds.

By controlling the amount of oxygen available to *Penicillium roqueforti*, and loose cheese curds, the ripening process was substantially modified. The growth metabolism of *P. roqueforti* is aerobic, therefore, controlling the oxygen allows ripening of the cheese curds to be controlled.

Packaging materials were chosen according to their gas transmission rates. Low-density polyethylene (LDPE) and Ethylene-vinyl acetate (EVA) were chosen for their ability to allow gases to permeate through the material.

Laminated aluminum foil (LAF) pouches were chosen because little, if any gases will transmit through the pouch. These pouches are capable of retaining gases for long periods of time.

"In-package" ripening of Blue cheese curds will not produce a cheese to be compared to that of commercial Blue cheese; but the loose curd product can be used in salad dressings, toppings, appetizers, etc.

Ideally, with this process, cheese may be able to ripen within packages during distribution or just prior to shipment. This could result in less warehousing space needed during the manufacturing and ripening of Blue cheese and enable more efficient utilization of storage space.

The possible elimination or reduction in growth of contaminant organisms, both pre-package and post-package is another important factor to be considered.

The research reported herein was directed toward an evaluation of the feasibility of this novel approach to ripen loose cheese curds.

LITERATURE REVIEW

The ripening of cheese is a complex phenomenon. The physical state and concentration of the original constituents as they exist in cheese, the enzyme systems involved (both intra and extracellular), along with added materials such as sodium chloride, and the physical environment in which cheese is placed, all influence the ripening process. The problem of elucidating what happens during ripening is complicated further because milk constituents are continually being changed by enzymatic action and by other chemical reactions. The process of ripening is generally evaluated by changes in flavor, appearance, body and texture (Morris et al. 1963).

Flavor

Cheese flavor is a blend of original milk constituents and fermentation products. Some constituents may remain until further changed by microorganisms or until specific enzymes are released from lysed bacteria. Products may accumulate, such as tyrosine, upon which no microorganisms or enzymes may be available to act (H.A. Morris, 1978).

The exact chemical nature of the flavor of any given cheese variety has not been completely elucidated; however, much information is available which provides a partial understanding of cheese flavor and the complicated reactions responsible for flavor formation (Harper, 1959).

Dulley (1976) developed a new method to accelerate flavor development. In this work, the author used cheddar cheese slurries as a source of enzymes and bacteria in cheese making. Regular manufacturing techniques were used until salting the cheese, at which time 600g of slurry (at 33C) was added and stored for approximately one week in plastic bags. Cheese to which slurries had been added ripened faster than normal cheeses.

In a study by Olson (1982) it was found that flavor enhancement was temperature dependent. An enzyme from Bacillus subtilis did not have an effect in cheese ripened at 43F but enhanced flavor development at 52 and 62F. This temperature effect is advantageous since cooling the enzyme treated cheese should stabilize flavor intensity at a desired level during distribution. Still, the necessity of using elevated temperatures during ripening could produce flavor defects. An analysis of various procedures to accelerate cheese ripening was made by a committee of the International Dairy Federation (IDF). The IDF suggested that elevated temperatures represented the most feasible method to accelerate flavor development in cheddar type cheeses under commercial conditions at the present time. The next most promising technique was adding selected enzymes, but this process needed further refinement.

A difficult problem in cheese flavor research is relating the objective chemical analysis to the subjective flavor evaluation. Although grading experts will usually agree as to the type of desired characteristic flavor of particular cheeses, there may be lack of agreement as to the degree of perfection of the flavor (freedom from other flavors) or as to its intensity. Therefore, it is difficult to obtain proof that a compound is a part of the desired characteristic flavor (Harper, 1959).

The rate of flavor development has been stimulated by producing a granular curd product, rather than the traditional loaf. Kondrup and Hedrick (1963) describe a method in which granular Blue cheese is ripened at elevated temperatures. It is claimed that satisfactory body and flavor equivalent to fully ripened conventional cheese can be obtained in a ripening period of 10 days.

Effect of mold on cheese curds

Like other cheeses of its type, Blue cheese is produced by introducing the mold spores of *P. roqueforti* or *P. glaucum* into the milk or curds before pressing. Air passages are then bored into the heavily salted cheese. The air induces the mold spores to grow vegetatively with a resulting spread of their greenish-blue mycelia. The penicillia molds are rich in proteolytic and lipolytic enzymes, and during the resulting ripening or curing these enzymes are released (Kosikowski,1977).

Growth of *P. roqueforti* inside cheese becomes evident about 8 to 10 days after the cheese is pierced (Foster et. al. 1957).

Development of mold is maximal in 30 to 90 days; by then the mold has grown throughout the spaces between curd particles and along holes made when the cheese was pierced (Marth, 1974).

Accumulation of carbon dioxide within the ripening cheese may reach a level inhibitory to good mold growth. This may be a possible reason for less mold growth in cheese ripened at the higher temperatures, which were otherwise still below the optimum growth temperatures for such species of mold (Peters and Nelson, 1961).

Effect of salt on cheese curds

Most organisms cannot survive salt concentration greater than about 6%, and essentially none survive a concentration of 10% (Morris, 1969). *P. roqueforti* can grow at salt concentrations up to about 16% brine. Usually a 10% brine is the concentration maintained in Blue-veined cheeses. This concentration discourages contaminating molds, yeast and bacteria from growing.

Kosikowski and Mocquot (1958) estimate the common salt (sodium chloride) content of Roquefort-type cheese to be between 3 and 4%, with a brine concentration of about 8%. Peters and Nelson (1961) used 8 to 10% (in brine) for their various experimental, 4 week old cheeses.

Increasing the percent of salt added to curds just prior to hooping resulted in increased moisture retention by such cheese, ripened 12 week old Blue cheese made from curd containing 1% added salt, based on weight of curd, excelled all other cheese in mold growth, texture, body and flavor scores. Cheese made from curd containing 2% added salt resulted in less moisture retention; it caused a brittle, weak or pasty body, and flavor defects.

The beneficial effect of adding 1.0 to 1.5% salt to curd prior to hooping observed in this study and others may be explained by the fact that the addition of salt to curd shifts the zone of insolubility of paracasein which appears at pH 4.5. This results in early firming of the curd particles and produces a more open textured cheese.

Babel (1953) claimed that high salt content (4%) and low oxygen tension inhibits growth of other molds in Blue cheese. Knez (1960) showed "conclusively" that inadequate salt and its slow penetration in to the interior of Niva were the main cause of so-called "Romadur" ripening, i.e., brown discoloration, absence of mold growth and objectionable taste. The salt level in the fresh cheese where this defect occurred was less than 0.75%, it appeared that this was one of the less heavily salted Roquefort types.

In Blue cheese manufacture, salting follows removal of the cheese from the hoops, which is followed in turn by skewering (Macquot and Bejambes, 1960). Lane and Hammer (1938) observed that delaying salting until 2 to 8 days after skewering could result in an unsatisfactory yellow color.

Goss et al. (1935) applied brine-salting over 2 days, followed by dry-salting over 4 days. Lane and Hammer (1938) applied 3 successive dry saltings over an 8 to 10 day period, incorporating 5.5% by weight of the cheese. This appeared to give more uniformity than the previous combination of brine and dry salting. This latter procedure appears to have fallen into disuse. It became normal practice to add part of this dry salt (1% by weight) to the curd with the mold powder (Peters and Nelson, 1960).

Work by Geurts et al. (1974) confirmed earlier work by McDowall and Dolby (1936) that about 90% of the water in cheese is free as a medium in which ripening occurs and as a solvent for salts. Salt concentration in the free water of cheddar cheese as made today for curing, may approach 6%. If milk salts are added to this, then the total ionic strength becomes an important factor in bacterial growth and enzyme activity. The effect of ionic strength, oxidation--reduction potential, and product inhibition need further examination.

Morris and Jezeski (1953) found that the lipase activity of P. roqueforti decreased with increases in the salt concentration--a 10 to 16% concentration causing a decrease of more than 60%.

The high activity and the amount of salt in the cheese caused a rapid demise of lactic starter bacteria so that only a few viable cells remained in cheese that was 2 to 3 weeks old, (Foster et al., 1957).

Acceleration of ripening

The time required for the natural development of internal mold veins is comparatively long, it may require over 6 months storage, during which there is much expense in the handling of the cheese and considerable loss of cheese weight by evaporation and mite attack. Economic stresses make it too expensive a process, so it is a general practice to inoculate the milk or curd with cultures of the mold, which develop in a much shorter period. This so-called "forced" blue cheese may have all the advantages of color and veining, with a coat free from mites, but it has not had has not had time to become as mellow. The mold grow before the proteins have been digested by the enzymes, even though they flavor it by the products of enzyme action, especially the hydrolysis of fats, the basic ripening of the curds is less. The rich flavor of a slowly matured cheese tends to be mellower, (Rogers, 1935).

Hedrick and Kondrup (1963) developed a method of quick ripening Blue cheese. The method was normal until after drainage of the curd, which was broken up into pieces not more than 1 inch in size, and placed on a fine mesh screen instead of being hooped. The curd was stored at 62F in a relative humidity of 95% for 6 days, during which it was salted and regularly stirred and then ripened for another 10 days. This method was claimed to be equal to 3 months normal ripening. The product was then stored in polyethylene bags.

Harte and Stine (1977), modified a technique for quick ripened blue cheese. This technique involved direct inoculation of mold into milk prior to addition of rennet, and rapid curing of the particular cheese curd in place of hooping, pressing, and aging. Ripening occurred in 7 days, in contrast to curing, up to 1 year using conventional methods.

Controlled atmosphere packaging

One use of controlled atmosphere packaging is as a method of packing perishable produce in specific gas mixtures to extend the shelf life of the particular product. This has great advantages to the retailer in allowing a full range of products to be kept on display for a period of time without running out of stock over the weekend. The extension of shelf life also allows the products to be packed in a central location providing economics of large scale over products being packed at individual retail outlets. In addition, packing in a central location releases the floor space needed for preparation and packaging and thus increase the sales area available. Controlled atmosphere packaging (CAP), involves containing a perishable product in a gas mixture chosen to prevent deterioration of the product. The gases are chosen to maintain both visual appearance and prevent microbial spoilage of the product as well as other deterioration. The gases commonly used in CAP are oxygen, carbon dioxide, and nitrogen, the proportions of each, varying according to the type of product being packed (Hanson and Duckworth, 1982).

When the natural atmosphere is removed from the surface of perishable foodstuffs, such as bacon, cooked meats, continental sausages, cheeses, etc., the deterioration of that product is substantially retarded well over its natural shelf life (Ross, 1982).

A variation of vacuum packaging is called gas flushing. Instead of removing the air from a pack and leaving the product in a vacuum, the evacuated air is replaced by a gas (usually carbon dioxide and nitrogen) which serves to protect, and possibly even enhance, the properties of the product. The process is used mainly for small, portion size packs for the product at point of sale (Anonymous, 1977).

Some dairies use gas flush packaging for bulk hard cheese, while others simply vacuum pack. Grated cheese packs benefit from gas flushing, by it preventing the separate pieces from sticking together (Anonymous,1977).

Among the advantages of flexible transparent vacuum or gas packaging are, the ability to protect a product against deterioration utilizing a low cost packaging medium, the ability to display the product itself with greater eye appeal because of the high degree of transparency, and the ability of the packer, by means of suitable printing, to identify himself with his product at the retail level. Various inert gases, such as nitrogen and carbon dioxide or mixtures of the same have been tested. In the case of cheese, an atmosphere of nitrogen was found to be suitable (Packaging Institute Papers, 1956).

Once the right gas mixture is established, the choice of film for a gas flush operation is very important. The film must allow oxygen to go into the pack in direct relation to the amount of oxygen used and carbon dioxide must permeate out as it is produced, to keep the atmosphere constant. The film must also allow water vapor loss, as pre-packaged produce lose water.

The whole future of this process relies on the development of the right film for products, as all products respire at different rates. Cheese wrapped in polypropylene under ordinary conditions normally last around 14-15 days. Packaged in a controlled atmosphere it can last for up to 4 weeks. The system has proved very successful since it was introduced (Hampton, 1983).

Gas Transmission

In the broadest sense, a package for a food material must perform three functions. First, it must physically contain the food product; second, it must maintain the quality of the food product, and third, it must be appealing to customers so that he or she will buy the product. In the first two of these functions, the permeability of the package plays a major role.

The term "permeability" is generally thought of as the passage of water vapor, and/or gases through protective films.

In the broader sense the term must include the passage through films of liquids such as water, fats, oils and volatile components possibly imparting flavor and odor to foodstuffs.

Water vapor and gas permeability are of primary importance in food packaging. It is safe to say that the successful packaging of any food product must take these two factors into account and in many cases the selection of a packaging material will depend primarily on one or both of these factors, (Charlton and De Long, 1956).

Factors affecting permeability

Both the gas and water vapor permeabilities of films 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.

All of these factors generally affect permeability. Those which affect permeability in a less direct manner are temperature, relative humidity, film thickness, characteristics of particular gases in association with specific films, and plasticizers. In the case of organic films, where solution permeability is of primary importance, an increase in film thickness results in decreased permeability (DeLong and Charlton, 1956).

Polyethylene's low moisture transmission is an important property, although the high gas rate may be a disadvantage for some applications, it is an advantage for packaging fresh produce, where escape of carbon dioxide and admission of oxygen prolong shelf life. Gas transmission rate decreases as temperature is reduced.

Shrink films generally provide close film contact with the cheese body resulting in minimal oxygen entrapment.

Very soft or non-uniform cheeses (mozzarella, pizza) in which entrapped oxygen may be a problem due to the irregularities of the cheese are commonly packaged in shrink films (Anonymous,1967).

Ionson (1979), in TABLE 1 described a method for the transfer of oxygen through packaging material for film ripened cheeses.

Table 1. OXYGEN BALANCE SHEET FOR FILM RIPENED CHEESES

OXYGEN GAIN	OXYGEN LOSS
A) Enclosed under film	E) Absorption by cheese (bacterial action reducing systems)
B) Leakage through defective seals,in overlaps, or end folds	F) Utilization by mold
C) Leakage through punctures or chafted areas in film	G) Balance available for mold growth and oxidation
D) Permeation through film	

In most cases, the water vapor permeability is considerably higher than the gas permeability. Two exceptions to this general trend are aluminum (laminated or not) and polyethylene. Aluminum foil is inert to water, so again the only mechanism which permeation occurs is through pinholes.

Polyethylene is more strongly hydrophobic than the other organic films so water vapor is quite insoluble in the film and hence penetrates it only to a limited degree. This particular property is of considerable value in the packaging of items which require a low moisture loss, while maintaining the ability to "breathe" with the passage of significant amounts of oxygen or carbon dioxide. The packaging of certain types of fresh produce is a good example of the requirements satisfied by this type of film (Charlton and DeLong, 1956).

Acidity

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

According to Coulter et al. (1938a) the pH of Blue cheese is influenced by the acidity of the cheese at setting and the acidity of the whey at dipping. If the acidity was too high during manufacture, the cheese developed an acid flavor, tough curd and crumbly body. Low acid cheese on the other hand, has soft curd and a tendency toward off flavors (Albert, 1974).

According to Hall (1936) the pH of Blue cheese shortly after manufacture was 4.6. The pH dropped to a minimum at about pH 4.5 at the end of about 15 days. During the next 15 days there was a rapid decrease in the hydrogen ion concentration of the cheese to pH 4.70. The acidity decreased slowly but uniformly as the cheese aged. The pH appeared to reach a maximum at about 4.70 after 24 hours. After salting, the pH increased rather rapidly until the cheese was pierced to admit air. Following piercing, the pH dropped from about 5.0 to 4.8, after several days the pH again decreased. Cheese was pierced on the 19th day at which time the pH was 5.01. On the 22nd day the mean was 4.77.

Growth of surface organisms

Microorganisms and enzymes are influenced by a) composition and structure of the medium or substrate, b) temperature and time, c) pH, d) salt concentration in the free water (ionic strength), e) water activity, and f) oxidation-reduction potential of the system. In addition to the common factors which influence microbial and enzymatic action, microbiological activity is influenced by competition and synergism among organisms and inhibition by accumulated metabolic products. Microbiologists are concerned with the identity of microorganisms in cheese ripening, sequence of appearance of demise during ripening, metabolic patterns, genetic manipulation of cheese-related microorganisms, and culturing techniques (H.A. Morris, 1978).

Many soft and semi-hard varieties of cheese commonly develop a sticky layer on the surface and is usually referred to as "slime". However, the microorganisms isolated from this mucilaginous layer have been assigned an important role in the curing of Limburger (Kelly, 1937) and Brick cheese (Langhus et al. 1945). Blue veined cheese, such as Roquefort or Minnesota Blue, also develop a characteristic surface growth. The development of this slime on blue cheese seems to follow a pattern. During the first 3 to 4 weeks, mold growth is accompanied by the development of a sticky surface growth. This gradually is replaced with a reddish orange, sticky slime (Hall et al., 1925 and Matheson, 1921). The slime developing on Blue cheese has been described briefly as consisting of yeasts, micrococci, and rod-shaped bacteria (Evans, 1918).

Initially, the slime consisted predominately of yeasts which decreased in numbers as ripening proceeded. The bacterial flora consisted of several types of micrococci, as well as rods, the latter seeming to predominate (Hartley and Jezeski, 1954).

The growth of the slime has been used as an index of proper ripening conditions (Hall and Phillips, 1925; Thom and Matheson, 1914). It also has been observed that its presence is associated with the best grades of cheese (Matheson, 1921).

Morris, et al., (1951) indicated that normally slimed Blue cheese developed a finer flavor and body than unslimed Blue cheese. Whether this was due to a direct action of the slime or to other factors was not determined.

Salt and pH effect on slime producers

The salt and pH tolerances of the typical organisms isolated from the slime were determined to seek possible explanation for their appearance at various stages during ripening. All of the organisms studied possessed a high tolerance to salt and grew at concentrations up to 15%. Several cultures grew at concentrations of 20% salt. Several strains of bacteria were sensitive to low pH, B. enthrogenes did not grow at pH 5.8 but did at pH 6.4,

B. linens and two micrococcus grew at pH 5.8, but not at pH 5.4. The pink Micrococcus sp. grew in a rather narrow pH range of 6.9 to 8.8. But it did not grow at pH 6.4 or 9.1.

Several unidentified yeasts grew at pH 3.1, but not at pH 2.6. Growth was somewhat limited up to pH 4.2. All of the organisms grew at pH 9.4 except the micrococci discussed previously. Initially, the pH of the slime rose, probably as a result of the action of yeast. Later, as the micrococci appeared, the pH decreased and then remained between 6.5 and 7.0. The pH of the cheese just beneath the slime of the Minnesota blue cheese was found to increase from 5.3 to 6.5 during the sliming period.

The slime was found to vary between 52 and 60% moisture during curing, the salt content varied from 3.1 to 5.0%. The cheese just beneath the slime had a moisture content of about 42 to 44% and a salt content of 4.0 to 5.2%, (Hartley and Jezeski, 1954).

The organisms present in the slime are highly salt-tolerant and enjoy mildly alkaline conditions. Many are inhibited below pH 5.8. Other researchers found similar results. (Kelly 1937 and Langhus et al., 1945).

It has been shown that factors such as pH, methods of salting, curing and packaging can affect ripening activity of organisms, and can cause color defects (in mold) and promote growth of contaminant organisms (Babel, 1953).

Packaging materials

In a study by Ionson (1979), package treatments influenced the extent of mold development on cheese. The development of mold was believe to be related to the presence of oxygen beneath the packaging film. Variables which affected the presence of oxygen were:

1) Barrier properties of the films
2) Incidence of flex-crack or pinholes
3) Extent of air entrapment
4) Gas production by the cheese
5) Wrinkles at the seal area

Prevention of wrinkles in the seal during packaging required that the material lie flat across the impulse bar and be free from crimping.

If the atmosphere within a package is oxygen deficient and/or carbon dioxide rich, the metabolism within a package of Blue cheese, will be suppressed.
Atmosphere metabolism is inversely related to storage life. The intensity of metabolism is evidenced by the respiration rate in some fruits. The rate of respiration is altered by changing temperature and partial pressure in the environment. Metabolism can be depressed in a number of ways; decreasing oxygen concentration, increasing carbon dioxide concentration, decreasing temperature, or any combination of the three (Kidd and West, 1974).

LDPE

Some of polyethylene's important functional characteristics include:

1) Protection because it is tough, flexible, water-vapor resistant, tasteless. odorless, non-toxic and practically chemically inert.

2) High degree of elongation and stretchability before break, prevents tear propagation and assists in giving polyethylene its notable toughness and long life.

3) From the standpoint of permeability to water-vapor, polyethylene is classed as a good barrier. Due to the fact that it is a homogeneous or monolithic material its water-vapor transfer rate varies inversely compared with the thickness. As the film becomes thicker the rate decreases. (Tibbets, 1956).

EVA

According to Hanlon, (1983) Ethylene-vinyl-acetate, (EVA) has properties, which vary according to the different percentages of components that are used. A blend containing 90% ethylene will resemble LDPE, whereas a 70-30 mixture will take on the physical characteristics of gum rubber.

LAF

Cage and Clark, (1980), described laminated aluminum foil pouches as providing superior barrier properties for a long shelf life, seal integrity, toughness, and puncture resistance. The material development took many years and resulted in some very sophisticated laminations.

An example is a polyester film/adhesive aluminum, foil/adhesive/polypropylene film. The polyester film is used for high temperature resistance, toughness, and printability, and is adhesive-laminated to aluminum foil for excellent barrier properties. The inner polypropylene film is adhesive-laminated to the aluminum foil and provides heat-seal integrity.

The printing of this particular laminate is applied to the "reverse" side of the polyester film, trapping the inks between layers to protect against scuffing. In the middle, aluminum foil is the key to a completely shelf stable food package, with no expensive freezing or refrigeration required. Aluminum foil is an excellent barrier to light, moisture, oxygen and microorganisms. On the inside, the polypropylene film performs two important functions.

1) It is inert and does not react to food, so that virtually the entire range of processed foods can be packaged in this one basic material.

2) It provides exceptionally strong heat seals that can withstand pressure and temperature demands which contribute to its shelf-life (Heintz, 1980).

EXPERIMENTAL PROCEDURE

Preparation of Cheese Curds

Loose cheddar cheese curds were obtained fresh from the M.S.U. Dairy Plant, prior to salting and milling. To reduce potential clumping or sticking together due to the high initial moisture content, the curds were cut into small cubes, approximately 1/4 inches wide. *P. roqueforti* spores, (5.80 grams and 11.60 grams) and salt (0%, 1%, or 2%) were then added and thoroughly mixed by hand into the cheese curds (5.0 pounds). All utensils and surrounding areas in contact with the cheese were sanitized with a diluted hypo-chlorite solution to reduce potential contamination.

The moisture content and the pH of the cheese curds were determined at the onset of the ripening period for each batch of cheese.

Water Vapor and Oxygen Transmission of LDPE and EVA

LDPE and EVA (area=0.0054m^2) were placed separately over the mouth of aluminum dishes which contained desiccant and sealed with molten wax. The assembly was placed in an atmosphere of constant temperature (100F) and humidity (85%).

The weight gain or loss of the assembly was used to calculate the rate of water vapor movement through the sheet materials (ASTM E96,1985).

A Mocon Oxtran 100 was used to measure oxygen transmission rates of LDPE and EVA (area=15cm). Castelletti and Soroka, 1979).

LAF was assumed to be an excellent barrier, therefore no permeability tests were performed.

Packaging and Storage of Cheese Curds

Prepared loose cheese curds were packaged (1/4 lb/pkg.) into previously constructed flexible pouches of LDPE, EVA and pre-formed laminated aluminum foil pouches (6"X 8 1/2").

These materials were chosen because of their flexible nature, differing ability to allow an influx of gases into the package, and ability to retain moisture.

The transparency of LDPE and EVA permitted the ripening process to be monitored without the need to open the package. LAF pouches did not allow transmission of light, nor the rapid influx of gases.
However, it did retain headspace gases long enough to ripen the cheese.

Cheese curds were packaged under ambient atmospheric conditions using LDPE and EVA pouches. Cheese curds were also packaged under controlled atmosphere conditions utilizing a gas flush of 30% oxygen and 70% nitrogen in LDPE, EVA and LAF pouches.

Curds packaged in ambient conditions (LDPE and EVA) were sealed with an impulse heat sealer. Curds packaged in LDPE and EVA under controlled atmospheric conditions were sealed with twist ties while scotch tape was used to seal LAF pouches. Each pouch was visually inspected for leaks, holes, wrinkles and any other seal imperfections.

Storage conditions were maintained at approximately 52F and 55-60% relative humidity (RH) for the duration of the study.

After the first 24 hours of storage, headspace gas analysis, pH and sensory analysis were performed on the cheese curds. These analyses were performed on a daily basis throughout ripening.

Visual changes in the cheese curds and obvious growth of contaminant organisms were noted throughout the duration of the study.

Package Environment

Initially packages were designed and filled under normal atmospheric conditions (approximately 21% oxygen and 79% nitrogen) utilizing a heat seal closure. Packages were also filled under controlled conditions within a glove box with an atmosphere composed of 30% oxygen and 70% nitrogen.

Periodically, gas samples were extracted from the glove box and analyzed using a gas chromatograph to ensure that the right concentrations of gases were maintained throughout the packaging procedure.

Cheese curds were packed under elevated levels of oxygen to determine whether or not the ripening rate of the cheese would be affected by increasing the initial oxygen level. Material thickness and package surface area can enhance or decrease the rate of transmission of gases into and out of the package. This could effect the rate of ripening. Therefore, careful control of material thickness and pouch surface area was maintained.

Using a hygrometer inserted into a package, initial and final relative humidity readings confirmed that the atmosphere within the pouches maintained a 90-95% relative humidity.

Salt Concentrations

Several different levels of sodium chloride (0%, 1% and 2%) were added to the non-ripened cheese curds prior to packaging. This was done to determine the effect on ripening of the spore inoculated cheese curds and the growth of contaminant organisms.

Mold Concentrations

The effect of inoculating the differing levels of *P. roqueforti* spores was evaluated by adding 5.80 grams or 11.60 grams of spores per 5.0 pound batch of curds. *P. roqueforti* spores were obtained in a powdery, freeze dried matter from Dairyland (Wisconsin).

Flow Diagram: Procedure of Packaging, Storing and Evaluating Loose Cheese Curds

Made pouches (6x8 1/2") from EVA (0.8 mil), LDPE (1.0 mil) and obtained pre-formed LAF pouches

↓

Applied rubber silicone on pouches for purposes of gas analysis

↓

Obtained fresh cheese curds from M.S.U. Dairy Plant

↓

Determined initial moisture content

↓

Used knife to cut curds into 1/4" cubes

↓

Determined initial pH

↓

Inoculated with desired amount of _P. roqueforti_ and salt

↓

Mixed curds, mold and salt thoroughly

↓

Packaged under ambient conditions 1/4 lbs/pouch and sealed

↓

Controlled atmosphere packaged 1/4 lbs/pouch and sealed

↓

Stored in controlled temperature and relative humidity chamber

↓

Determined moisture content (after 24 hours)

↓

Began daily analysis of pH, gas headspace and monitored color changes (after 24 hours)

↓

Determined final moisture content of cheese at end of study

After the cheese curds had been prepared and stored, packages were divided into several different batches. As outlined below, the packaging material, amount of salt and/or mold, and the atmosphere in which the curds were packaged determined which batch the curds belonged.

Table 2. Batches of packaged loose cheese curds

Low Density Polyethylene	Ethylene Vinyl Acetate
BATCH #1 (Control) 0% mold, 0% salt	BATCH #7 (Control) 0% mold, 0% salt
BATCH #2 1/2% mold, 0% salt	BATCH #8 1/2% mold, 0% salt
BATCH #3 1% mold, 0% salt	BATCH #9 1% mold, 0% salt
BATCH #4 1% mold, 1% salt	BATCH #10 1% mold, 1% salt
BATCH #5 1% mold, 2% salt	BATCH #11 1% mold, 2% salt
BATCH #6 (Controlled Atmosphere) 1% mold, 2% salt	BATCH #12 (Controlled Atmosphere) 1% mold, 2% salt

Laminated Aluminum Foil (Controlled Atmosphere)
1% mold, 2% salt

ANALYTICAL PROCEDURES

Sampling

Each day of the ripening process, a different package of curds from each material (LDPE, EVA and LAF) was obtained from the control chamber and used for sampling.

Moisture Content

The moisture content of the cheese curd was determined by the vacuum oven method (AOAC, 1975). Determinations were made in triplicate and performed initially and at appropriate stages of this study.

pH

pH measurements were determined by using an Orion digital pH/mV meter equipped with a glass electrode (Standard Methods for Examination of Dairy Products, 1972). The measurements were made by inserting an electrode into blended cheese samples for 5 minutes. Measurements were taken initially and continued on a daily basis throughout the ripening period for each batch of cheese.

Headspace Analysis

Headspace analysis, using gas chromatography was utilized to determine the concentration of carbon dioxide and oxygen in the packaged cheese curds.

Prior to packaging the curds, Dow Corning silicone rubber was deposited on each pouch. After the rubbery material was cured, a needle (syringe) was inserted through the patch without causing the pouch to tear or leak.

The syringe was filled to the desired volume (approximately 1cc). The contents of the syringe were then injected into the gas chromatograph (GC) (Packaging 427 Lab Manual, 1982).

Samples were taken in duplicate after 24 hours and on daily basis throughout the ripening period for each batch of cheese.

Hygrometer Analysis

A hygrometer was used to record the relative humidity on the inside of the packaged cheese curds. A sensor was positioned in a LDPE pouch so as not to interfere with the ripening activity, then sealed with Dow Corning silicone rubber.

Measurements of the relative humidity inside the package were taken after the first 24 hours and on the last day of the ripening process.

Sensory Analysis

An experienced cheese judge scored and rated individual packages of cheese. Flavor and color were evaluated. Scores ranged from 1-5, (1, least favorable to 5, most favorable).

Each package of cheese was judged at the onset of the study and continued until all samples had been evaluated. Results were not statistically evaluated, but were used to indicate acceptability.

Microbial Observations

Visual inspection of each pouch was made regularly, to detect presence of undesirable contamination by molds, yeasts or bacteria during the ripening period.

These observations were made after 24 hours and continued daily throughout the study. Visual examinations were instrumental in detecting mold growth or spoilage organisms.

RESULTS AND DISCUSSION

pH

According to Marth (1974), the pH of commercial blue cheese initially was at 4.5 to 4.7 and increased from 6.0 to 6.25 after 2 to 3 months of ripening.

Growth of *P. roqueforti* becomes evident about 8 to 10 days after the cheese is pierced (Foster et al.1957). Marth (1974) stated, that proteolytic enzymes from the mold act to soften the curd and thus to produce the desired body in the cheese. Some components of blue cheese flavor may result from the proteolytic action.

pH of curds packaged in LDPE

The pH of control cheese curd, BATCH 1 (0% mold and 0% salt) can be seen in FIGURE 1. Initially, the pH was low, probably due to the activity of starter culture producing lactic acid. Since the curds contained no mold or salt the pH did not appreciably change throughout the 10 day storage period. A slight increase near the end of storage may have been due to the presence of contaminant organisms.

The pH of cheese curds, BATCHES 2 and 3, (1/2% mold, 0% salt and 1% mold, 0% salt respectively), can be seen in FIGURE 2. The initial pH in both batches, were approximately the same, until DAY 7 when the curds containing 1/2% mold had a pH increase to 6.2. This increase coincided with color development which indicated proteolytic activity. The increase in pH from the initial minimum to the maximum probably occurred as a result of proteolysis by the starter.

culture and growth of _P. roqueforti_. Bryant and Hammer (1940) reported that a rise in the pH of cheese is due to the proteolytic action of the mold on the whey proteins, and might be responsible for the change in the color of the spores. On DAY 8, the pH decreased to 5.3 and remained relatively constant until removed from storage. Coulter et al. (1938), noted a slow decrease in pH to approximately 5.7 after 4 to 9 months of ripening. He attributed this decrease to an accumulation of free fatty acids (FFA) in the cheese. Even though BATCH 3 contained double the mold concentration, the increase in pH was not as great as in BATCH 2. Improper mixing, or defective package sealing may have attributed to the slower rise in pH. The pH of cheese curds, BATCHES 3, 4, and 5 (1% mold and 0% salt, 1% mold and 1% salt and 1% mold, 2% salt respectively) can be seen in FIGURE 3. Initially, the pH in BATCHES 3 and 5 were approximately the same, with BATCH 4 having a slightly higher pH. This may have been due to variation in manufacturing of the cheese.

As the pH increased to 6.7 in BATCH 4, the color of the

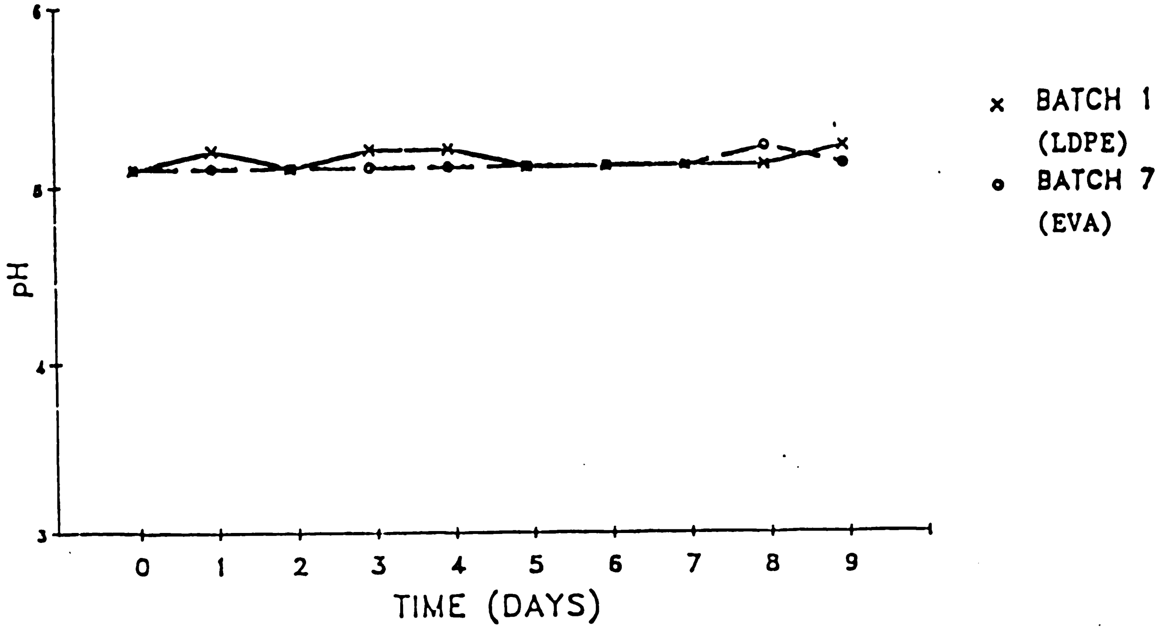

Figure 1. Change in pH during ripening of Blue cheese made with 0% mold spores and 0% salt, packaged under ambient conditions in LDPE and EVA

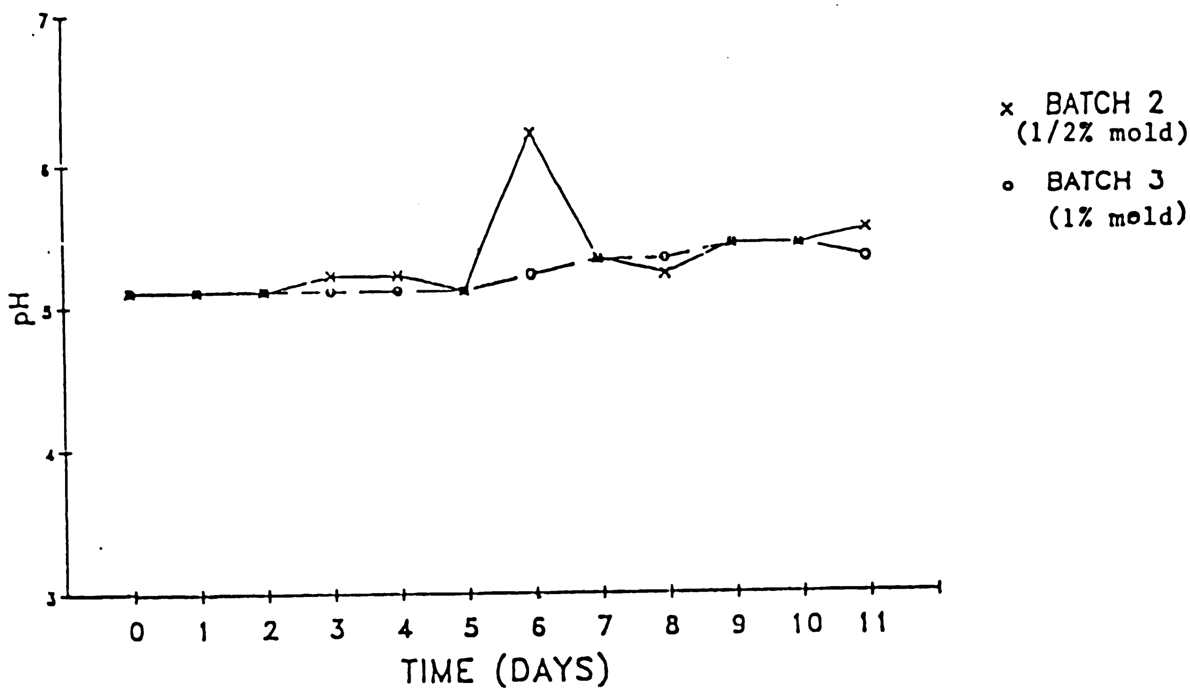

Figure 2. Change in pH during ripening of Blue cheese made with 1/2% and 1% mold spores and 0% salt, packaged under ambient conditions in LDPE

curd changed from blue highlights to a definite blue color. The largest increase in pH usually coincided with the first visible sign of mold growth, and thus proteolysis. The next day, DAY 12, the pH began to decrease, but the color remained the same throughout the study. Cheese held in storage beyond 10 days usually showed gradual a decrease in pH throughout the 18 day storage period. This decrease may have been due to the increased production of volatile acids. Kosikowski (1966) stated, that a low concentration of salt is stimulatory to spore germination. In some Blue cheese manufacturing procedures up to 0.3% salt is added to the milk at renneting or hooping. pH differences between BATCH 4 and 5 indicated that the higher salt concentration in BATCH 5 may control or inhibit activity of micro-organisms. Stadhouders (1960) found that the inhibitory effect of salt was more pronounced in the range of 1-2% than at higher concentrations, and that acid production in Roquefort cheese diminished as the salt content increased from 3.0-3.9%.

The pH of cheese, BATCHES 5 and 6 (1% mold, 2% salt, packaged in 21% oxygen and 30% oxygen respectively), can be seen in FIGURE 4. Throughout most of the storage time BATCHES 5 and 6 had similar color and pH. On DAY 10, the pH (BATCH 5) began to decrease and then leveled off while the pH of cheese in BATCH 6 continued to gradually increase. Higher levels of free fatty acids (FFA) released by the mold lipase system could have been responsible for the

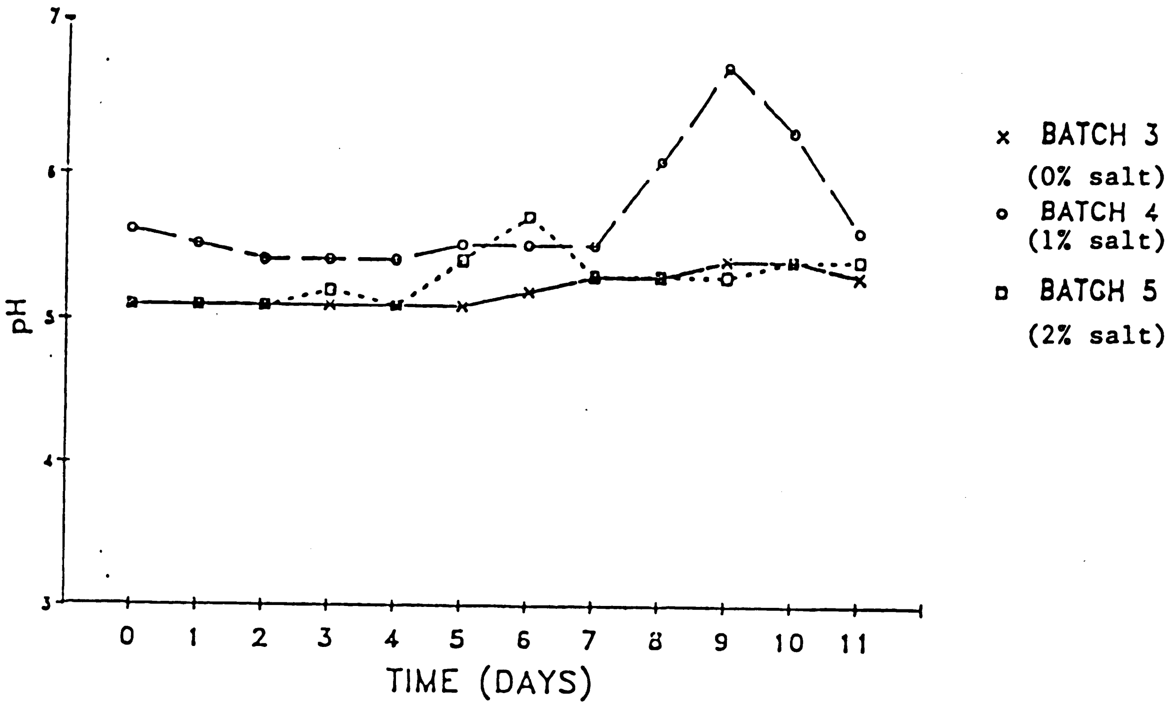

Figure 3. Change in pH during ripening of Blue cheese made with 1% mold spores containing different levels (0%, 1% and 2%) of salt, packaged under ambient conditions

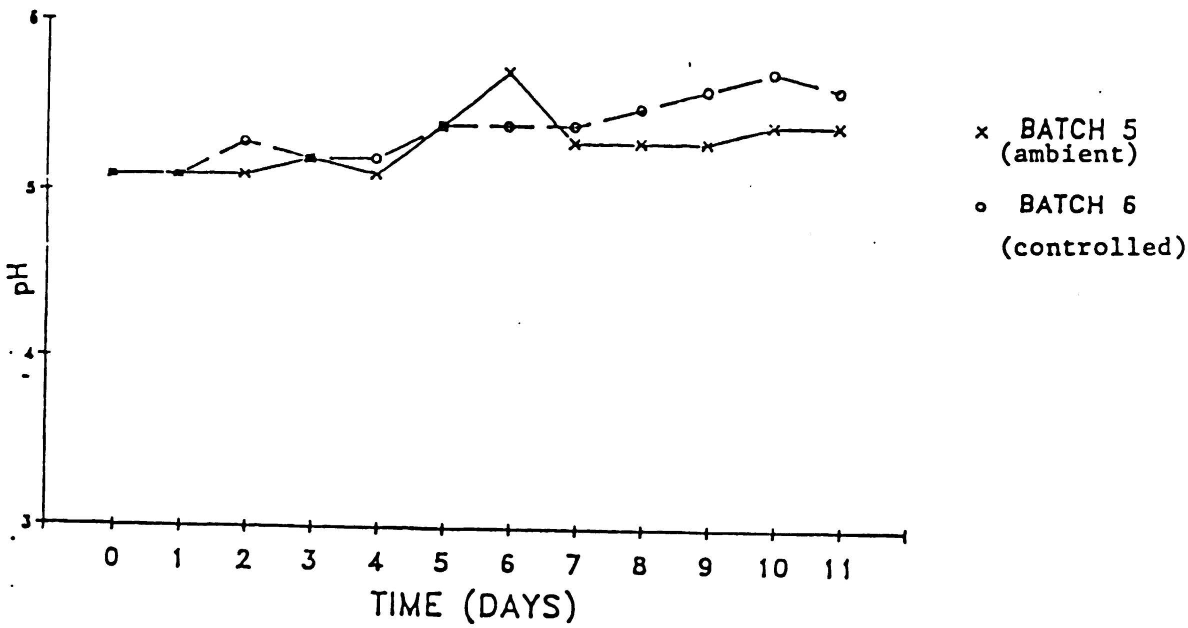

Figure 4. Change in pH during ripening of Blue cheese made with 1% mold spores and 2% salt, packaged under ambient and controlled conditions in LDPE

stabilization or slight decrease of pH in BATCH 6. In addition to similar pH values, both had similar gas headspace concentrations as seen in FIGURE 9. After only 24 hours of storage , the initial oxygen concentration of both batches decreased tremendously. As expected, concentrations of carbon dioxide and oxygen demonstrated an inverse relationship, as oxygen decreased carbon dioxide increased. Changes in gas headspace profiles, (FIGURE 9, BATCH 5 and 6) indicate mold growth/activity as early as DAY 5. During this time, carbon dioxide was relatively high and oxygen was relatively low. This respiration was a major indicator that P. roqueforti was utilizing the oxygen present in the contained packages at a rapid rate. Curds color changed from white to gray; also pH valves began to increase.

Thibodeau and Macy (1942), and other workers have shown that the mycelium of P. roqueforti contains proteolytic enzymes. Bryant and Hammer (1940) reported that grey discoloration was most common in over-ripened, blue-veined cheeses and that the discolored patches had an ammonia odor and higher pH than the normal parts of the cheese. Changes in pH or headspace, occurred approximately on the same day. This illustrated a definite correlation between pH and gas headspace and also indicated that ripening was occurring. Gas headspace concentration, pH and color changes are a result of the germination of P. roqueforti and also indicated when ripening will occur.

In addition, the permeability of LDPE promotes the opportunity for mold development because it allows the passage of oxygen and carbon dioxide readily.

pH of curds packaged in EVA

The pH of control cheese curd, BATCH 7 (0% mold and 0% salt), can be seen in FIGURE 1. Initial pH values were low, probably due to the release of lactic acid. Throughout storage, there were no significant changes in pH. Final pH readings were similar to initial readings. As expected the control batch of curds remained relatively inactive and produced no observable changes.

The pH of cheese, BATCHES 8 and 9,(1/2% mold, 0% salt and 1% mold, 0% salt respectively) can be seen in FIGURE 5. The initial pH of both batches was approximately the same, BATCH 8 showed a significant increase on DAY 7. This increase in pH coincided with definite blue color. Regardless of whether or not the curds contained 1/2 or 1% mold, the cheese ripened at approximately the same rate. Differences in mold concentration did not significantly cause variation in color.

The pH of cheese curds, BATCHES 9, 10 and 11 (1% mold and 0% salt, 1% mold and 1% salt and 1% mold, 2% salt respectively) can be seen in FIGURE 6. Initially, the pH's (BATCHES 9 and 11) were approximately the same, while the initial pH for BATCH 10 was substantially higher. The higher pH could have been a result of pre-contamination or

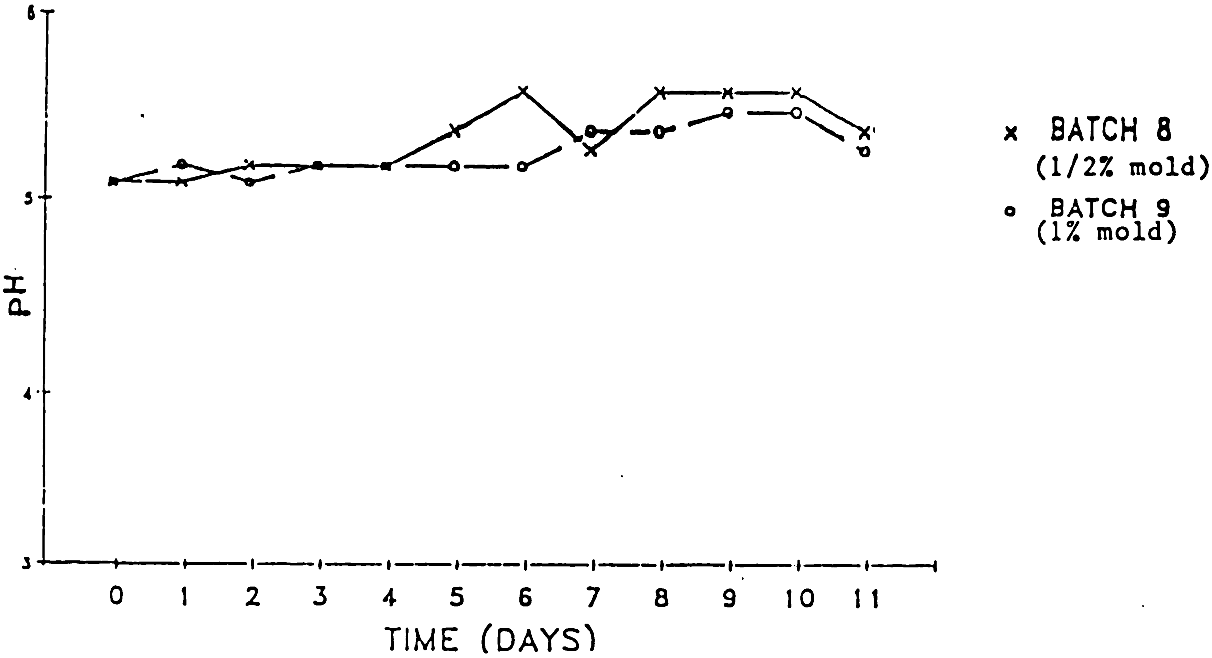

Figure 5. Change in pH during ripening of Blue cheese made with 1/2% and 1% mold spores and 0% salt, packaged under ambient conditions in EVA

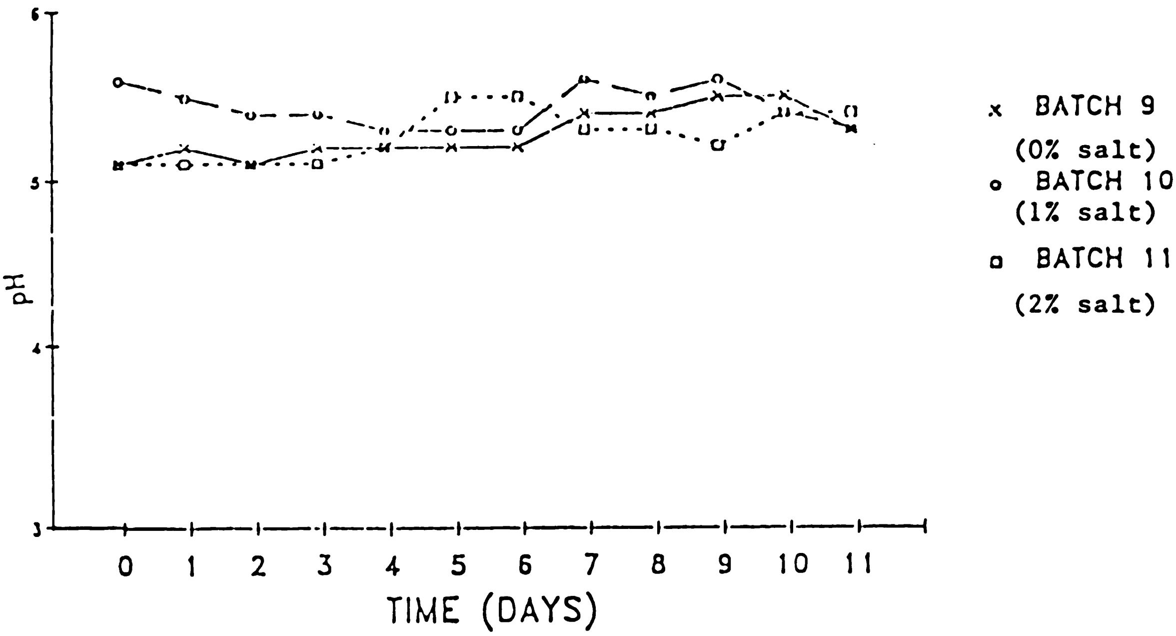

Figure 6. Change in pH during ripening of Blue cheese made with 1% mold spores containing different levels (0%, 1% and 2%) salt, packaged under ambient conditions in EVA

a variation in the manufacturing process.

By DAY 5, all 3 batches had pH values approximately the same, for BATCHES 9 and 10 the pH remained relatively close throughout the storage period. pH in BATCH 11 increased significantly on DAY 6 which correlated to change in color. Soon thereafter the pH decreased. The change in pH was dependent on salt concentration with gradual change occurring in BATCHES 9 and 10, with a more sudden pH change occurring in BATCH 11. The amount of salt could have a prohibiting or enhancing factor, depending on the concentrations. Morris and Jezeski (1953) showed that an increase in salt concentrations, decreased acids present in Blue cheese.

The pH of cheese curds, BATCHES 11 and 12 (1% mold, 2% salt, packaged in 21% oxygen and 30% oxygen respectively), can be seen in FIGURE 7. Initial and final pH values in both batches were approximately the same, there were no significant differences in pH or color between the 2 batches. The different concentrations of headspace oxygen affected the pH of the curds on different days. On DAY 10, BATCH 12 had the lowest oxygen concentration, which corresponded to the cheese curds attaining definite blue color and change in pH. The lowest oxygen concentration for BATCH 11 was on DAY 5, which coincided with initial change in color and change in pH. Cheese curds appeared to progressively ripen in either atmosphere even though headspace gas concentrations were

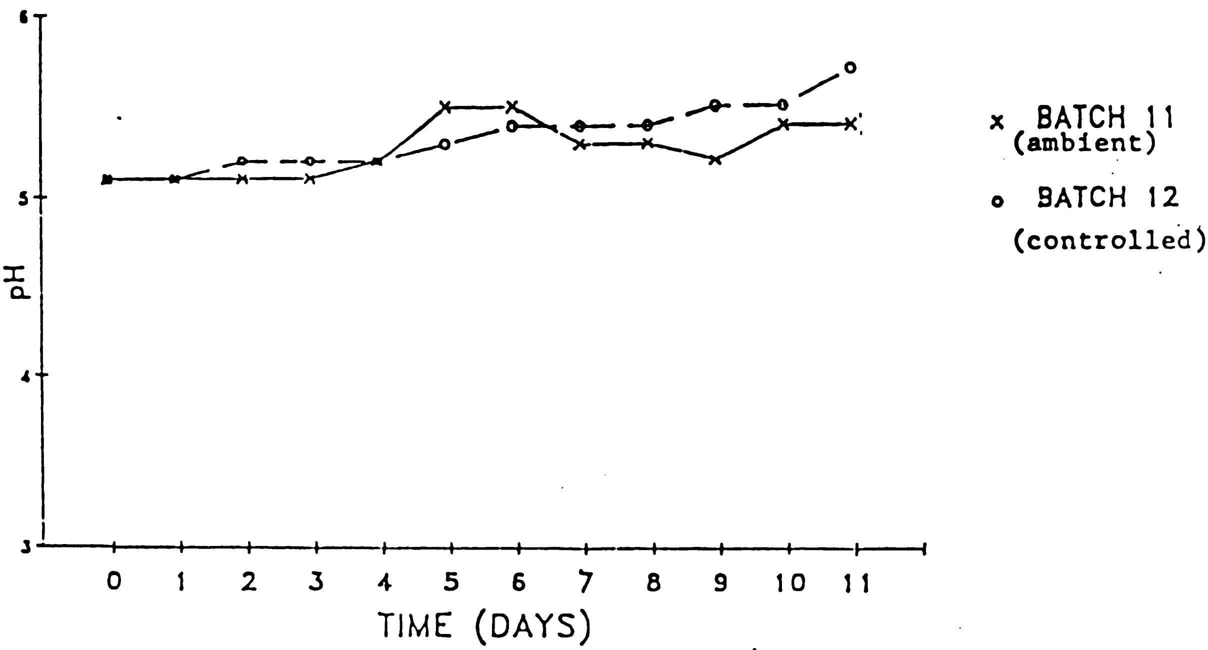

Figure 7. Change in pH during ripening of Blue cheese made with 1% mold spores and 2% salt, packaged under ambient and controlled conditions in EVA

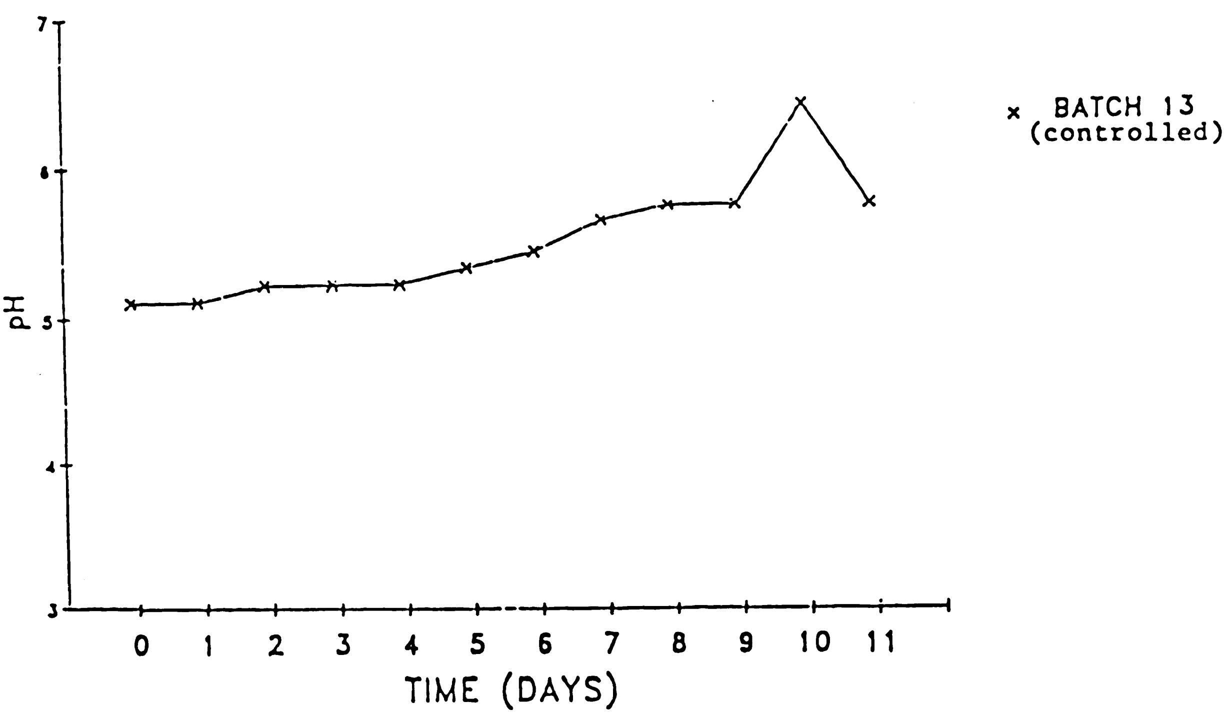

Figure 8. Change in pH during ripening of Blue cheese made with 1% mold spores and 2% salt, packaged under controlled conditions in LAF

significantly different (FIGURE 10).

Different mold concentrations (1/2 and 1%) did not significantly alter the pH of the curds. Mold concentration does not necessarily influence the pH of cheese curds.

Table 3 compares oxygen and moisture transmission rates between EVA and LDPE. Although EVA has a higher permeability rate than LDPE in some instances curds packaged in LDPE ripened sooner than curds in EVA. The two polymers share some similar characteristics, i.e. stretch, cling, and static, but their physical/chemical properties are quite different. The barrier properties of LDPE provided an "in-package" atmosphere conducive to ripening the curds faster than EVA.

Table 3. OXYGEN AND MOISTURE VAPOR TRANSMISSION OF EVA AND LDPE

POLYMER	OXYGEN TRANSMISSION RATE $cc/100in^2/24hrs$	MOISTURE VAPOR TRANSMISSION RATE $g\ mil/100in^2/24hrs$
ETHYLENE VINYL ACETATE	2220	35.5
LOW-DENSITY POLYETHYLENE	1930	25.5

<u>pH of curds packaged in LAF</u>

The pH for cheese in BATCH 13, (1% mold and 2% salt, packaged in 30% oxygen can be seen in FIGURE 8. Initially the pH was relatively low, approximately the same as BATCH 6 and BATCH 12, which were also packaged under a controlled atmosphere. From DAY 6 through DAY 9, the pH of the cheese curds increased to a greater extent than either BATCH 6 or 12. The increase in pH corresponded to initial color changes, and an increase in carbon dioxide gas headspace. Although curds packaged in aluminum foil were completely covered with mold and had substantial blue color, they were also very powdery and the texture was slightly dry.

The even distribution of mold and blue coloring was not necessarily an indication of good or acceptable flavor. According to Rogers, (1935) "forced" (accelerated) blue cheese may have all the advantages of color and veining, but it has not had time to become mellow. LAF is an excellent oxygen barrier material. Packing the cheese in an initial high oxygen concentration allowed the mold to grow very rapidly. This rapid growth, did not allow the enzymes enough time to break down flavor producing constituents, thereby, leaving an unpalatable blue cheese. Rogers (1935) also stated "forced" blue cheese allows mold growth before the proteins have been sufficiently digested, although the cheese is flavored by enzyme action, especially the split-up of fats, the basic ripening of the curd is less. The rich flavor of a slowly matured cheese, where mold has naturally developed, tends to be mellower.

BATCH 13 revealed that the manufacture of "in-package" Blue cheese in LAF pouches failed to produce a cheese of typical flavor. Blue cheese prepared by "in-package" ripening under controlled atmosphere conditions packaged in LAF was considered sensorially unacceptable under the conditions used in this study.

Charlton and DeLong (1956) found that LAF is inert to water, therefore the only mechanism through which permeation occurs is through pinholes. Polyethylene and EVA is more strongly hydrophobic than the other organic films, therefore, water vapor is quite insoluble in the film and hence penetrates only to a limited degree. This particular property is of considerable value in the packaging of items requiring a low moisture loss, while maintaining the ability to "breathe" with the passage of significant amounts of oxygen or carbon dioxide.

Gas headspace of controlled atmosphere packages

All of the controlled atmosphere packages were stored for 14 days. Initial results indicated BATCH 6 had the highest oxygen gas headspace, while BATCH 12 had the lowest initial carbon dioxide headspace. Final results indicated BATCH 12 had the lowest overall percentages of oxygen and carbon dioxide. BATCH 13 recorded the highest overall percentage of both gases. The differences found in BATCHES 6, 12 and 13 could possibly be due to experimental error; the differences could also be due to the permeability of the particular packaging materials, and defective seals.

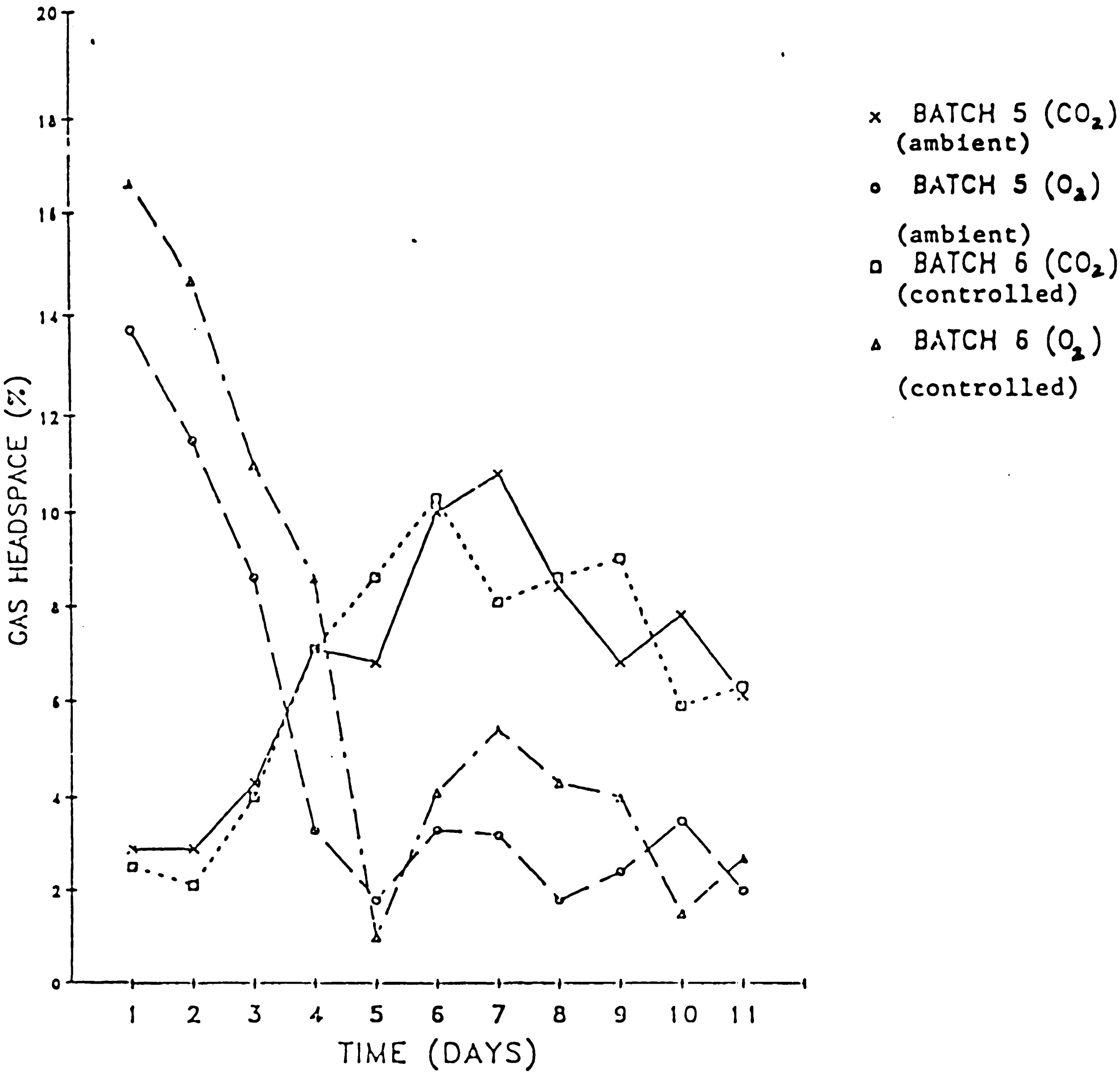

Figure 9. Change in gas headspace during ripening of Blue cheese containing 1% mold spores and 2% salt packaged under ambient and controlled conditions in LDPE

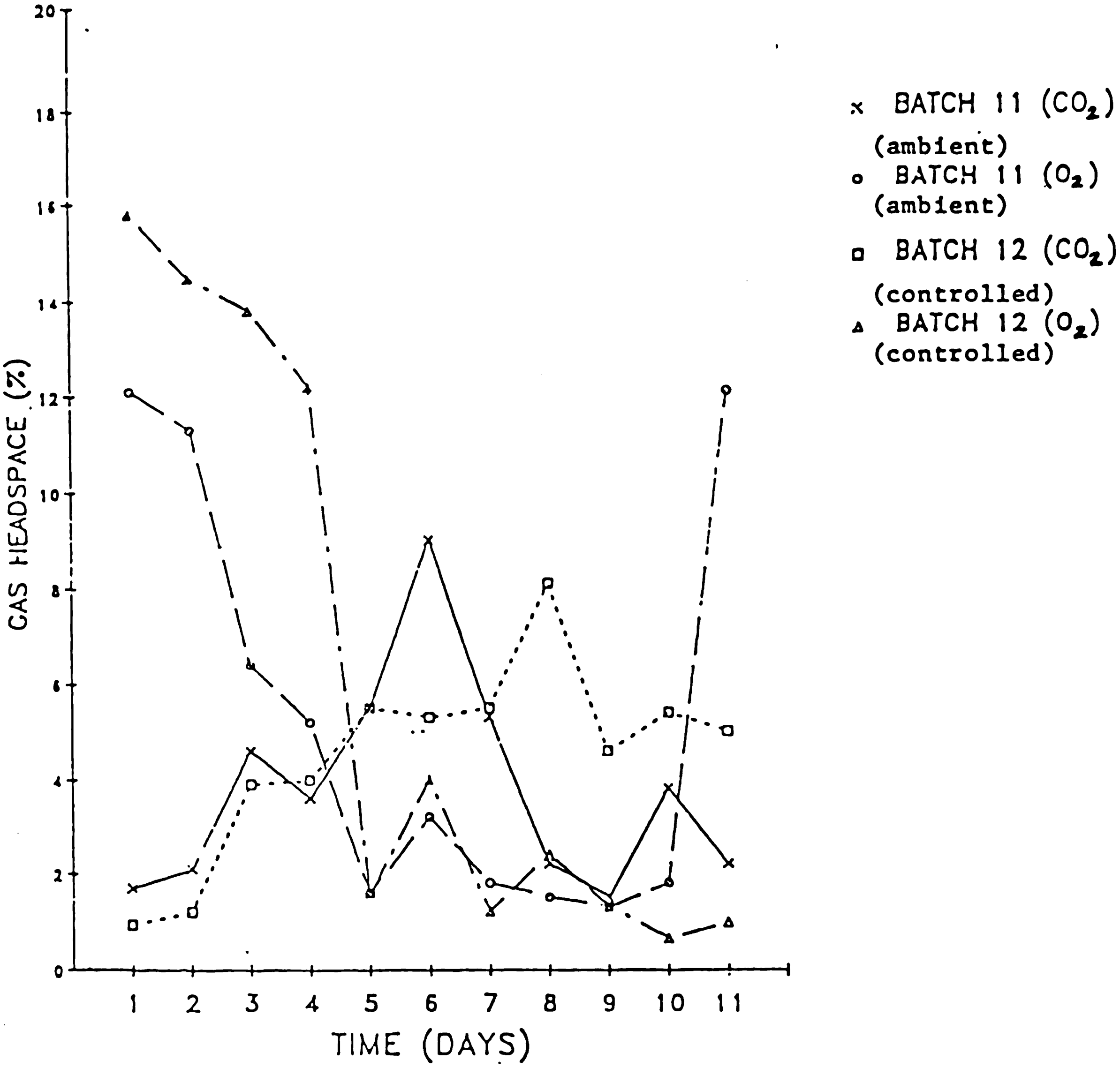

Figure 10. Change in gas headspace during ripening of Blue cheese containing 1% mold spores and 2% salt, packaged under ambient and controlled conditions in EVA

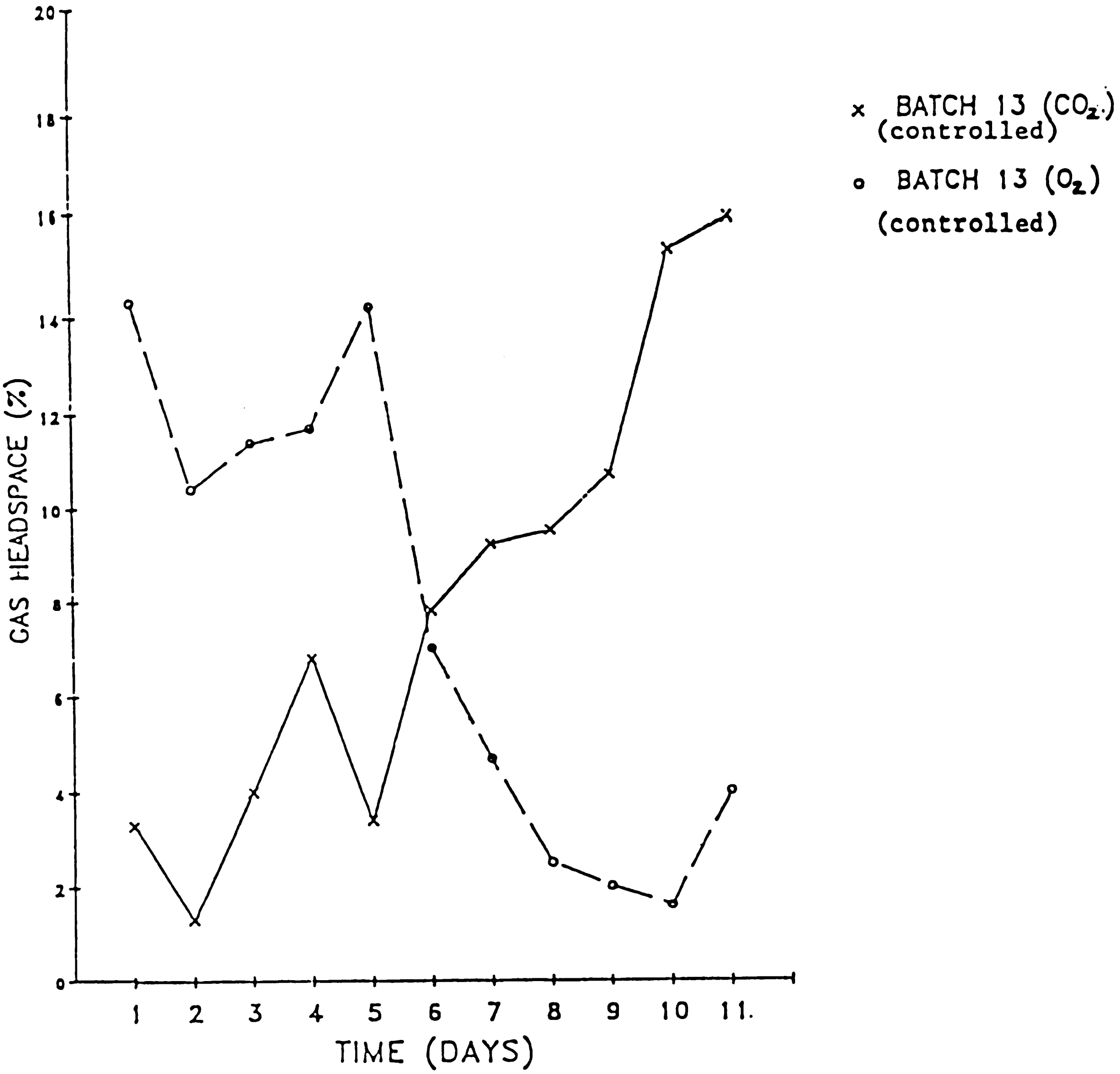

Figure 11. Change in gas headspace during ripening of Blue cheese containing 1% mold spores and 2% salt, packaged under controlled conditions in LAF

Moisture content

The amount of moisture in cheese influences its rate of ripening, pH, flavor, and nutritive value. Moisture content is important to the consumer and to the cheese maker.

Ability of cheeses to be stored is closely related to the moisture content, acidity, and several other factors that influence keeping quality. Generally, the higher the moisture and the lower the acidity, the shorter the shelf life (Marth, 1974).

According to the classification of natural cheeses by hardness and moisture content, commercial blue cheese is considered to be a semi-soft, high moisture (45-55%) cheese (Considine 1982).

Kristoffersen (1978) explained that the after processing quality of cheese depends essentially on the nature of the internal and external environments of the cheese during curing and ripening. Important factors of the internal environment include: moisture content, which ranges between 32 and 53% depending upon variety; lactic acid content, which ranges from 0.8 to 2.1%; the pH value, which may range from 4.7 to 5.1; and salt content, which ranges from 0.5 to 6.0% among other factors.
The internal environment depends on many constituents added to the starting milk.

In this study, the moisture content for each batch of cheese was determined at 3 stages: (1) prior to inoculation and packaging, (2) 24 hours after inoculation and packaging, and (3) upon completion of the ripening period.

The moisture content for all batches of cheese is shown in TABLE 4.

For all batches of curd, moisture ranged from 40.0% to 47.4% , from the beginning to the end of storage, respectively. Moisture content was dependent upon the manufacturing process and degree of ripening.

The interrelationship of pH and water activity is of practical importance. As the mold grew, the pH increased. Initially, when the moisture content was high, there was not much mold production, but as the curds began to dry out, mold growth increased. When moisture content was at its lowest level, mold production was usually at its highest.

Although salt and mold slightly influenced the moisture content, different permeability rates of the packaging materials had a greater influence on moisture.

Moisture content of cheese packaged in LDPE

As expected, there was no significant ripening activity in control BATCH 1 (0% mold and 0% salt), therefore, the moisture content decreased only slightly during storage.

BATCHES 2 and 3 (1/2% mold, 0% salt and 1% mold, 0% salt respectively) had an initial decrease in moisture content. During the storage period BATCH 3 lost more moisture than BATCH 2, which resulted in a lower final moisture content. Perhaps the greater concentration of mold in BATCH 3 used more moisture, initially, for ripening the curds. Although BATCH 3 resulted in a slightly lower final moisture content than BATCH 2, the moisture content of BATCH 3 was comparable to the other batches.

Moisture contents for BATCHES 3, 4, and 5 (1% mold, 0% salt and 1% mold, 1% salt and 1% mold, 2% salt respectively), were similar.
Initially, BATCH 4 had the highest moisture content with BATCH 5 slightly less. BATCH 3 showed the greatest decrease in moisture over a 24 hour period and lowest moisture content by the end of the study. BATCH 4 had the highest initial and final moisture content. Batches that initially had low moisture contents also had low final moisture contents, and batches that had initially high moisture contents had high final moisture contents. Salt levels did not seem to have a major influence on the moisture content of the different batches.
Although the moisture contents for all batches that contained mold were very similar, the differences in mold level seemed to slightly influence moisture content.
In general, batches that contained 1.0% mold spores had a lower final moisture content, than batches with 1/2% of mold. This indicated that the mold utilized more water

during ripening.

Initially the moisture content for BATCH 5 (1% mold, and 2% salt packaged under atmospheric conditions of 21% oxygen) and BATCH 6 (1% mold and 2% salt packaged under accelerated conditions of 30% oxygen and 70% nitrogen), were very similar. After 24 hours of storage, BATCH 5 had slightly less moisture than BATCH 6. Throughout the study, BATCH 6 retained slightly more moisture than BATCH 5. The moisture content of BATCHES 5 and 6 were comparable to other batches of cheese. The higher oxygen atmosphere had no significant bearing on the moisture content.

Moisture content of cheese packaged in EVA

The moisture content in control BATCH 7 (0% mold and 0% salt) decreased slightly during storage. Some moisture may have been lost through permeation of the packaging material. Since EVA has a higher water permeability rate than LDPE or LAF, some moisture loss from the curds could be attributed to the permeability of EVA.

BATCHES 8 and 9 (1/2% mold, 0% salt and 1% mold, 0% salt respectively) had similar moisture contents from the initial stages of ripening through the end of storage. Both batches experienced the greatest moisture loss during the first 24 hour period. Although both batches had similar moisture contents, BATCH 9 lost slightly more moisture, probably due to the higher mold concentration.

Moisture contents for BATCHES 9, 10 and 11 (1% mold, 0% salt and 1% mold, 1% salt and 1% mold, 2% salt respectively) varied slightly. Initially, BATCHES 9 and 10 had very similar moisture contents, while BATCH 11 had the lowest initial moisture content. Final moisture levels for BATCHES 9 and 11, were quite similar. Although it was anticipated that BATCH 11, because it contained more salt, would lose more moisture than BATCH 9. The mold and salt combination in BATCH 11, was ideal to promote ripening, however, the moisture content was expected to decrease appropriately. Although mold did grow, the growth was not accurately reflected in the final moisture content. The moisture determinations for BATCHES 9 and 11 were very close.
Perhaps BATCH 11 retained more moisture than necessary to facilitate ripening, whereas, BATCH 9 did not.

The moisture contents for BATCH 11 (1% mold and 2% salt packaged under ambient atmospheric conditions of 21% oxygen) and BATCH 12 (1% mold and 2% salt packaged under accelerated conditions of 30% oxygen and 70% nitrogen), were not much different.

It was determined that moisture content was not contingent on the initial oxygen level in this study. Although the accelerated environment induced a faster ripening curd, the mold spores apparently did not require any significantly higher level of water.

Moisture content of cheese packaged in LAF

In comparison to the other controlled atmosphere packaged batches, (BATCHES 6 and 12), BATCH 13, had the highest initial moisture content. During a 24 hour storage period, all three batches lost moisture, with BATCH 13 losing the least amount of moisture, and BATCH 12 the most. End of study results indicated that BATCH 12 lost the most moisture overall. Cheese curds packaged in laminated aluminum foil protected the cheese from moisture evaporation and, therefore, retained most of its moisture. The use of LAF retained curd moisture and promoted mold growth, thus inducing ripening. This enhanced mold productivity to the point that cheese curds which were ripened in LAF were much drier in comparison to curds ripened in either LDPE or EVA. It was observed that curds in LAF pouches appeared "over ripened" due to the abundance of color.

Acceptability of curds based on moisture content

EVA, has a higher water permeability rate, and produced curds with a soft/mushy texture, and slightly less mold growth and color were produced.

The permeability rate of LDPE allowed sufficient moisture to remain in the package, which resulted in cheese of good texture and color. Cheese packaged in LDPE was considered to be closer to commercial Blue cheese.

Whether curds are ripened in LAF pouches and become too dry and powdery, or packaged in EVA and contain too much moisture, either extreme will result in a product unacceptable to the consumer.

The composition of mold ripened cheeses 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 only BATCHES 6, 7, and 13 would be acceptable. The remaining batches are only slightly under the standards. Therefore, Federal Standards may present problems that would affect the marketability of this cheese.

Table 4. Moisture content of curds packaged in LDPE, EVA and LAF pouches

VARIABLES	LOW-DENSITY POLYETHYLENE			ETHYLENE-VINYL ACETATE		
	Prior to inoculation and packaging	24 hours after inoculation	End of study	Prior to inoculation and packaging	24 hours after inoculation	End of study
0% MOLD 0% SALT	BATCH 1 46.6%	46.6%	46.2%	BATCH 7 46.6%	46.4%	45.9%
1/2% MOLD 0% SALT	BATCH 2 47.1%	44.2%	41.2%	BATCH 8 46.8%	42.8%	40.3%
1% MOLD 0% SALT	BATCH 3 46.1%	42.3%	40.2%	BATCH 9 47.1%	42.3%	40.0%
1% MOLD 1% SALT	BATCH 4 46.8%	44.9%	41.3%	BATCH 10 47.0%	44.6%	41.5%
1% MOLD 2% SALT	BATCH 5 46.5%	43.1%	40.4%	BATCH 11 46.4%	42.6%	40.4%
CONTROLLED ATM 1% MOLD/2% SALT	BATCH 6 46.7%	42.0%	42.0%	BATCH 12 47.2%	44.5%	40.9%

	LAMINATED ALUMINUM FOIL		
CONTROLLED ATM.	Prior to inoculation and packaging	24 hours after inoculation and packaging	End of study
1% MOLD 2% SALT	BATCH 13 47.4%	45.5%	42.6%

FIGURE 12. Hygrometer records relative humidity inside a package of curds ripened in LDPE

Sensory evaluation

Sensory evaluation is one of the most vital tests that can be performed on any food product. Food products which are intended for commercial consumption should always be evaluated a priori.

In this study the packages of cheese curds were evaluated for flavor, color, texture and appearance. Flavor is a composite of sensations, of which taste and odor are important components.

W.J. Harper, (1959), stated that a difficult problem in cheese flavor research is relating objective chemical analysis to subjective flavor evaluation.

Scores for flavor and color ranged from 1 (the least acceptable) to 5 (the most acceptable). Scoring was done by an experienced cheese judge.
The closer the cheese curds resembled commercial Blue cheese the higher the scores.

Color and flavor of curds packaged in LDPE

Tables 5 through 10 present color and flavor scores of cheese curds packaged in low-density polyethylene. During initial ripening activity the curds began to have a moldy appearance, with yellow curds turning slightly green with gray specks. The curds required an average of seven days before blue color development was evident.

Curds which contained 0% salt and 1% mold tasted better and had better color than batches with salt.

Although salt is added to improve the flavor of cheeses it did not have that affect on these particular batches of curds. Salt is also added to suppress the growth of microorganisms capable of spoiling cheese. Curds with 2% salt had no visible signs of growth by spoilage organisms.

Although some batches (i.e. BATCH 4) received above average color scores, the flavor scores were below average. Therefore color and flavor were determined to be independent variables.

The texture of the curds were firm for approximately 10 days, but a slimy film appeared on the surface of some batches; then the curds became soft and mushy, possibly due to spoilage organisms. As the batches began to spoil, sensory scores decreased, pH values increased and moisture content decreased.

Curds packaged in controlled atmosphere showed initial changes in color and flavor sooner than the other batches, definite blue color also developed sooner. Even though curds became over-grown with mold, and very powdery, the texture remained firm throughout storage. The 2% salt possibly discouraged spoilage organisms from growing. pH values were stable. The higher oxygen levels had a definite impact on flavor development and on the rate that the cheese curds turned blue. The accelerated conditions encouraged mold to sporulate and according to flavor scores, resulted in an acceptable tasting "in-package"

TABLE 5. Sensory Evaluation of control curds packaged in LDPE, containing 0% salt and 0% mold

BATCH 1

DAY	COLOR	FLAVOR
1-10	1.0	1.0

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TABLE 6. Sensory Evaluation of curds packaged in LDPE, containing 0% salt and 1/2% mold

BATCH 2

DAY	COLOR	FLAVOR
1	1.0	1.0
4	1.5	2.0
5	2.0	2.0
7	2.5	3.0
10	2.0-2.5	2.5
12	2.5	3.0
13	2.5	2.5-3.0

TABLE 7. Sensory Evaluation of curds packaged in LDPE, containing 0% salt and 1% mold

BATCH 3

DAY	COLOR	FLAVOR
1	1.0	1.0
4	1.0-1.5	1.0
6	2.0	2.0
7	2.0-2.5	2.5
9	3.5-4.0	3.0
13	4.0-5.0	2.5-3.0
14	4.0-5.0	3.0

==

TABLE 8. Sensory Evaluation of curds packaged in LDPE, containing 1% salt and 1% mold

BATCH 4

DAY	COLOR	FLAVOR
1	1.0	1.0
6	1.5	1.0
8	1.5-2.0	1.0-1.5
10	3.0	1.5
12	3.5	1.5-2.0

TABLE 9. Sensory Evaluation of curds packaged in LDPE, containing 2% salt and 1% mold

BATCH 5

DAY	COLOR	FLAVOR
1	1.0	1.0
6	1.0-1.5	1.0-1.5
8	2.0	2.0
9	2.5-3.0	2.0
11	3.5-4.0	2.5-3.0
12	3.5-4.0	3.0

TABLE 10. Sensory Evaluation of curds packaged in LDPE, under Controlled Atmosphere, containing 2% salt and 1% mold

BATCH 6

DAY	COLOR	FLAVOR
1	1.0	1.0
4	1.5	1.5
6	2.0	2.0
9	3.5-4.0	3.5-4.0
11	3.0	3.0-3.5
14	4.0	3.5-4.0

ripened Blue cheese.

Color and flavor of curds packaged in EVA

Tables 11 through 16 present color and flavor scores of cheese curds packaged in ethylene vinyl acetate. With initial ripening of these curds slight hints of blue or gray hues developed. The texture was fuzzy/hairy until curds became powdery.

Curds which contained 0% salt and 1/2% mold, tasted better than some of the other batches that contained salt and 1% mold. This batch also produced enough color to be favorably compared to other batches that contained 1% mold. The lack of salt did not have a negative influence on the taste, in fact, the peppery/ sharp taste that makes Blue cheese desirable was enhanced.

Scores for curds with 1% salt and 1% mold remained below average from the beginning until the end of storage; none of the scores exceeded 2.0. Improper mixing or pre-package spoilage organisms could be responsible for the low scores. This batch was considered unpalatable.

Batches which contained the least and the most amount of salt were considered favorable. Curds with 0% salt allowed flavor to become distinct; 2% salt discouraged initial spoilage organisms and produced an acceptable tasting curd.

In this particular case, curds packaged in controlled atmosphere did not produce definite blue color as

TABLE 11. Sensory Evaluation of control curds packaged in EVA, containing 0% salt and 0% mold

BATCH 7

DAY	COLOR	FLAVOR
1-10	1.0	1.0

==

TABLE 12. Sensory Evaluation of curds packaged in EVA, containing 0% salt and 1/2% mold

BATCH 8

DAY	COLOR	FLAVOR
1	1.0	1.0
4	1.5	1.5-2.0
6	3.5	2.5
8	3.0	2.0
10	4.0-5.0	3.5
13	4.0	3.5

TABLE 13. Sensory Evaluation of curds packaged in EVA, containing 0% salt and 1% mold

BATCH 9

DAY	COLOR	FLAVOR
1	1.0	1.0
5	1.0-1.5	1.0
6	2.0-2.5	2.0
8	3.5	2.0-2.5
10	3.0-3.5	2.5
12	4.0-5.0	3.0
14	4.0-5.0	2.5-3.0

==

TABLE 14. Sensory Evaluation of curds packaged in EVA, containing 1% salt and 1% mold

BATCH 10

DAY	COLOR	FLAVOR
1	1.0	1.0
9	1.0-1.5	1.0-1.5
10	1.5	1.0-1.5
11	1.5-2.0	1.5
12	1.5-2.0	1.5-2.0

TABLE 15. Sensory Evaluation of curds packaged in EVA, containing 2% salt and 1% mold

BATCH 11

DAY	COLOR	FLAVOR
1	1.0	1.0
7	1.5	2.0
9	2.0	2.5
11	2.5	2.5
12	3.0-3.5	2.5

TABLE 16. Sensory Evaluation of curds packaged in EVA, under Controlled Atmosphere, containing 2% salt and 1% mold

BATCH 12

DAY	COLOR	FLAVOR
1	1.0	1.0
4	1.5	1.5
7	2.0	2.0
8	3.0	3.0
11	3.5	3.0
14	4.0	3.5

quickly as curds packaged in ambient conditions. But as ripening progressed, mold eventually grew and the curds became excessively powdery. Accelerated conditions did not significantly improve flavor, but curds received above average scores, and would appeal to consumers. Near the end of the study, the texture changed from firm to soft. The 70% nitrogen used to help control the package's atmosphere did not prevent spoilage organisms from occurring.

Kosikowski and Brown, (1973) found nitrogen to be less effective than carbon dioxide in repressing spoilage organisms and maintaining flavor freshness.

End of storage results revealed changes in texture and consistency. The curds became soft and mushy; this was the first macroscopic indication of spoilage organisms. All curds packaged in EVA became soft or mushy by the end of storage. Of the three packaging materials used in this study, EVA has the highest water-vapor transmission rate.

Color and flavor of curds packaged in LAF

Table 17 presents, color and flavor scores for cheese curds packaged in LAF. As early as DAY 4, color scores improved significantly, with slight improvements in flavor. Above average color scores were achieved and maintained throughout storage, while flavor scores remained average until the end of storage. Curds packaged in this controlled atmosphere did not become soft or mushy. However, the curds became excessively powdery and dry. Even distribution of mold and good blue color could easily account for the high scores in color, but it was determined that high color scores did not necessarily dictate high flavor scores. Perhaps the curds turned blue too rapidly, and the mold did not have the opportunity to properly ripen and mellow the curds, therefore flavor did not fully develop.

TABLE 17. Sensory Evaluation of curds packaged in LAF, under Controlled Atmosphere, containing 2% salt and 1% mold

BATCH 13

DAY	COLOR	FLAVOR
1	1.0	1.0
4	2.5	1.5
6	3.0-3.5	1.5
8	4.0	2.0
10	4.0	2.5-3.0
14	4.0	2.5

SUMMARY

A good quality Blue cheese can be manufactured by a controlled atmosphere "in-package" ripening process. The time involved in ripening the cheese curds is substantially less than that for commercially ripened cheese. "In-package" ripening will allow loose cheese curds to ripen within flexible pouches during distribution or just prior to shipment. This would result in less warehousing space needed during the manufacture and ripening of Blue cheese, and enable more efficient utilization of storage space.

This process also reduces the opportunity for contaminant organisms to infiltrate both pre-packaged and post-packaged curds.

The "in-package" ripened Blue cheese did not produce cheese comparable to commercial Blue cheese; but the loose curds could be used in salad dressings, toppings, appetizers, and sauces, etc.

Different concentrations of _Penicillium_ _roqueforti_ spores contained in the cheese curds, did not significantly increase the rate of ripening.
The different percentages of salt (0%, 1% or 2%) added to the curds affected flavor.

The curds which contained the highest and the lowest percentages of salt had higher flavor scores. The curds

with 1% salt tasted bland and undesirable.

The flavor of the curds was not dependent on color. In some batches high color scores were given, while receiving low flavor scores.

Controlled atmosphere packaging accelerated mold growth. Batches of curd packaged under a controlled atmosphere had enhanced color scores, but failed to significantly enhance flavor.

Gas headspace and pH were monitored daily to observe changes in the curds during ripening. Visible evaluation was used to detect contaminant organisms.

During ripening headspace profiles indicated decreased oxygen and increased carbon dioxide. pH values increased as the mold grew.

The amount of mold slightly influenced the initial moisture content of the curds. Usually 1% mold utilized more moisture during ripening than 1/2% . Different salt levels and controlled atmosphere did not influence moisture. Final moisture content percentages were approximately the same for all batches.

As the curds ripened, moisture was used. The use of different packaging materials could have affected moisture loss through water-vapor transmission, improper seals or defective material.

Appearance and texture of the curds were most influenced by moisture content but color was least effected. After approximately 10 days of storage, curds quality began to deteriorate and sensory scores decreased.

All three packaging materials: LDPE, EVA, and LAF utilized in this study provided the necessary barrier from outside spoilage organisms. LDPE and EVA allowed oxygen to permeate their walls. LAF retained sufficient gases within its pouches to facilitate ripening. Overall, curds packaged in LDPE resulted in a higher quality (appearance, consistency and texture) blue cheese. Curds in EVA were too moist and too dry in LAF.

It has been shown that "in-package" ripening of Blue cheese can be manufactured, but like anything in its infancy, further development is required.

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