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The role of propionibacteria in the split defect of Swiss cheese

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The role of propionibacteria in the split defect of Swiss cheese

by

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To Gretchen, Marc, and Erin

INTRODUCTION

Emmental, Swiss, or Swiss-type cheeses are hard rennet-cheeses characterized by their sweet flavor and gas holes or "eyes". Swiss cheese is an old variety claimed to have originated in the valley of the Emmental river, Canton Bern, Switzerland, probably in the middle of the 15th century. There are various types of cheese of later origin much like Swiss cheese, and with some of these the manufacturing procedure is quite different. Swiss-type cheeses are generally intended to have a sweet flavor and eye formation, although with some a semisoft body is desired. Swiss cheese made in the United States is properly called "domestic Swiss cheese" but because of extensive production and popularity, the term "Swiss cheese" has become commonly used and accepted.

Swiss cheese is a product of microbial fermentation of milk constituents and the quality of the finished cheese depends upon the growth and metabolic activity of the natural microflora of milk or of selected microorganisms added to the milk during manufacture. Essential microorganisms include Streptococcus lactis and/or Streptococcus cremoris, Streptococcus thermophilus, Lactobacillus spp., and a Propionibacterium spp. To achieve the desired complex and intricate interrelationships between the body, texture, and flavor characteristics of Swiss cheese, it is necessary to maintain a delicate balance between these various groups of microorganisms. This can be accomplished by manipulating pH, temperature, and salt concentration at various stages of manufacture

and ripening, thus encouraging the desired fermentations to occur at appropriate steps of the manufacturing process.

In conventional Swiss cheese, the curd is pressed into flat wheel shapes and with the resulting rind and greater surface to mass ratio produce a very different system from that found in rindless block Swiss cheese. Without the protective flexible film used in rindless cheese there is a significant loss of moisture during and after rind formation. J. B. Stine of Kraftco Corporation (123) developed rindless block Swiss cheese in the early 1940s and production has increased from about 49 million lb in 1940 to approximately 144 million lb in 1970 (129). Swiss cheese now ranks as the third most important variety of cured cheese in terms of total production volume in the United States, exceeded only by Cheddar and Mozzarella. It is presumed that over 90% of the U.S. production of Swiss cheese is in the rindless form (102).

The basic control of eye development depends upon the evolution of proper body and texture of the cheese curd, both initially and throughout making and curing. The description "proper body" means establishment of sufficient elasticity to permit ready expansion of eyes without attendant defects; at the same time, considerable firmness is necessary to withstand the physical and chemical forces that are active during curing. "Proper texture" implies the presence of a desirable number of faults, openings, or areas of insufficiently knit curd that will subsequently determine

the number and location of the developing eyes. The production, spacing, and size of eyes in Swiss cheese depend upon classical gas laws and on the solubility and behavior of the gas in a gel structure leading to saturation. For an eye to form in the cheese, some starting nuclei such as ash particles are necessary, and the cheese serum must become saturated with carbon dioxide. Furthermore, the gas generation is required at an optimum rate. The important and usually desirable gas-forming microorganisms are Propionibacterium spp. The usually undesirable gas formers belong to the genera Escherichia, Aerobacter, Clostridium, a few Lactobacillus and Bacillus, certain yeasts, and enterococci.

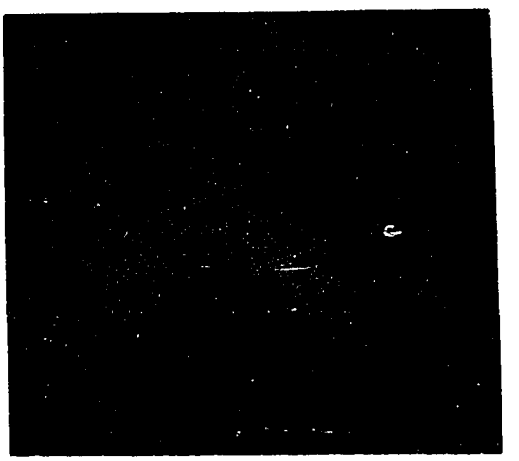
One of the least understood defects that occurs in Swiss cheese is the formation of fissures or cracks called glass, gläsler, or splits (Figure 1). This phenomenon appears in some lots of Swiss cheese receiving additional curing in the finished cooler after removal from the warm room. It is found most frequently in aged cheese. There usually are no undesirable flavors, but such cheese is difficult to slice efficiently for retail sale. The growing popularity of small-sized consumer packages has made the splitting of Swiss cheese an important economic problem, especially since the defect tends to occur indiscriminately. Ultimately, this defect must be related to lack of curd plasticity or to some abnormality in the curing fermentation.

This investigation was undertaken to study the role of propionibacteria in the occurrence of splits and to further study

Figure 1. Photographs of typical checks and splits in Swiss cheese

SPLIT and CHECKED

SPLIT



CHECKED

several related factors as to the cause of the split defect in Swiss cheese. These related factors include: (a) studies of the growth rate, production of carbon dioxide, and growth at low temperatures of propionibacteria in pure culture; (b) studies of the growth rate, production of carbon dioxide, utilization of lactate and sugars, and the effect on proteolysis by propionibacteria in Swiss cheese; and (c) studies of the metabolism and enzyme systems of propionibacteria as related to activity at low temperatures. Another approach involving the effect of packaging films to the problem of splits in Swiss cheese was investigated.

LITERATURE REVIEW

Splits and Their Occurrence in Swiss or Emmental Cheese

Carbon dioxide production and the formation of eyes

It was assumed by early investigators that if bacterial action was the cause of gas evolution in cheese, bacteria in sufficient numbers to produce the required amount of gas must be strictly localized about an opening in the cheese body (18). This certainly would be the most straightforward supposition to make, and, if true, it would seem that a comparison of the flora about the eyes against the flora in other parts of the cheese would lead at once to the discovery of the causative organisms. Such comparisons have, however, not furnished the striking results one would expect. Instead, many researchers (17, 18, 36, 103, 133) have shown that there is an unequal distribution of propionibacteria in Swiss or Emmental cheese and that they are not localized about the eye.

None of the artificial media devised so far approximate exactly the relative power of cheese to preserve a more or less constant hydrogen ion concentration with the consequent extension of growth and control of enzymic action. Nor do artificial conditions always simulate cheese in furnishing the proper degree of anaerobiosis. Thus, an organism, or its liberated enzymes, may be able to produce in cheese much more carbon dioxide than in artificial media, and, as the limits of growth and action are reduced

by artificial media, large differences in gas-producing power may become narrowed to such an extent that the powers of different organisms appear the same.

Orla-Jensen (91) concluded that the eyes of Emmental cheese were not caused by the same agents that produced swelling, or by yeasts or obligate anaerobic bacteria. He stated that they were produced by the same bacteria responsible for normal ripening, that is, lactic acid-producing organisms, and that the gas, which is the immediate cause of eyes, was not produced from milk sugar but from nitrogenous substances. He believed that, under certain conditions, the lactic-acid organisms of cheese formed traces of carbon dioxide from nitrogenous substances and that these small amounts were the cause of eye formation. Von Freudenreich and Orla-Jensen (138) found that lactic acid in Emmental cheese was fermented according to the following equation:

$$3CH_3CHOHCOOH \longrightarrow 2\ CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$$

The carbon dioxide liberated by the fermentation was said to be the cause of eye formation.

Sherman (116), in 1921, was one of the first workers to show that the essential organism for the production of eyes and the characteristic sweetish flavor of Swiss cheese belonged to the genus Propionibacterium. He isolated and studied these propionic-acid bacteria and found them to differ slightly in some characters

from the several varieties of propionic-acid bacteria which had been described in the literature.

Clark (18) reviewed the literature on eye formation in cheese and found little or no evidence that the eyes of Emmental cheese were strictly localized at points of excessive bacterial growth. He concluded that the gas produced in the cheese separated in aggregates, the locations of which had no necessary relation to the point where the gas was produced. Rapid gas production was considered to result in the formation of numerous small eyes, and slow gas production in the formation of large eyes.

Propionibacteria are now known to be responsible for the distinctive sweet flavor of Swiss cheese, but the indirect contribution of streptococci and lactobacilli to flavor has yet to be elucidated. Propionibacteria contribute much of the carbon dioxide that forms the eyes of normal Swiss cheese. Given the proper conditions, however, any microorganism that can produce sufficient gas, whether it be carbon dioxide, hydrogen, or other gas, can form eyes in Swiss curd. The number and location of the eyes will depend upon the presence of weak points in the curd that will permit and encourage the collection of sufficient gas to form eyes. Aside from intrinsic interest, determination of the factors which influence the size and spacing of the eyes is of direct practical importance inasmuch as present day merchandising requires many uniformly distributed, medium sized eyes.

Frazier and Wing (37) found that, in experimental cheese, an

excess of propionic-acid culture usually produced an overset cheese. (Probably the most common defect encountered in Swiss cheese grading is the overset condition which means too many eyes per block or section. The overset condition may occur throughout the cheese block in a uniform manner, or may be localized in specific areas). Excessive growth of propionic-acid bacteria, especially in the later stages of ripening, tended to cause glass and checking. (Checks are small cracks or fissures considered to be the precursors to splits in cheese). When moderate amounts of propionic-acid culture were used, the number of eyes formed, the time of beginning eye formation, and the time from completion of eye formation were independent of the number of propionic-acid bacteria added. Eye formation took place in some cheeses when less than 100 propionic-acid bacteria per gram were present at the beginning of eye formation, and little or no increase in numbers had taken place 1 week later. Such cheeses usually were not as sweet as those with more propionic-acid bacteria.

Flückiger and Reber (30), in testing Emmental cheese for carbon dioxide content by the Robertson method (108), found that the cheese samples varied widely both in carbon dioxide content and capacity to absorb the gas. Koestler (70) was able to show that only a fraction of the carbon dioxide produced during the degradation of lactic acid was retained in the cheese. Most gas escaped into the atmosphere.

The application of X-rays for observing the development of

eyes in Emmental and other cheese with eyes is an interesting development recently accomplished (114, 131). It is possible that X-rays could aid the manufacturer in watching the progress of eye formation and development and the development of possible defects.

Once the process of eye formation is complete the production of gas does not always terminate. Flückiger and Hostettler (29) studied conditions favoring the renewal of gas production in Emmental cheese. They found temperature to be important when the ripening room rose above 15 C even when the cheese was 6-months old. They also determined that higher moisture cheese produced gas more rapidly and that a period of storage at 0 C was ineffective in checking an incipient tendency for continuing gas production.

Splits in Swiss cheese

The increase in popularity of small-sized consumer packages and presliced portions has made splitting an important economic problem. Many theories have been advanced to explain the spasmodic occurrence of this defect but none have been truly acceptable. Statements to the effect that splits or "glass" are caused by too-acid and too-fat milk, or poor whey drainage inducing a brittle condition, only account for a minor portion of the defect. Quite frequently, the most acceptable cheese will develop this defect. It is found most often in cheese aging in the finished cooler after removal from the warm room.

In 1905, Decker (20) published the observation that with too-sweet milk, such as may be obtained in the cool fall months, it was difficult to get a good cook of the curd and that such cheese would have a pasty texture lending to the production of gläsler cheese (splits).

Hanson et al. (45) indicated that the lack of acid development, low salt content, and large loaves of cheese tended to encourage the development of late gas leading to the production of splits in Brick cheese. They stated that although these factors were important they were not necessarily the only ones involved in the production of this defect.

Van Beynum and Pette (132), in 1943, investigated the formation of various types of eyes in Edam and Gouda cheese and found that gas formed when the pH of the cheese was low produced splits in the cheese, whereas, at a higher pH, round eyes appeared. Propionic-acid bacteria were found in all cheese, but in greatly varying numbers. Their primary interest was to determine the nature of eye formation when fermentation occurred with butyric-acid bacteria, lactate-fermenting bacteria, or propionic-acid bacteria.

Reiner et al. (104) studied the formation of slits in Gruyére cheese and related them to tension on the cheese body. They were able to show paths of tension occurring particularly when propionic fermentation started at a late stage of ripening. They found that transference of the cheese from warm to cold storage

also may cause slits through thermic contraction.

Oehen (85) investigated the pH of a large number of Emmental cheeses from various factories in different areas and related pH to the incidence of glasler defect. He also compared the pH and defect incidence of cheeses produced in 1965 to 1967 to those produced 30 years earlier and found the incidence much higher when the cheese had a pH above 5.15. He concluded that there was no cause for concern when overripening of the cheese milk occurred even if relatively large inocula were used to ripen the milk.

Gardet (39, 40) found that none of 20 Emmental cheeses made with the addition of 8 to 15% water during various stages of the in-vat treatment exhibited the slit eye defect, whereas, 11 of 20 negative control cheeses did exhibit this defect.

Stadhouders and Veringa (120) described and determined the cause of some new texture and flavor defects in Gouda cheese. The cheeses had many eyes and a large number of large and small cracks in the later stage of ripening. They determined that these defects had a bacterial cause and were derived from use of rennet contaminated with undesirable microorganisms.

Frazier and Wing (36, 37) believed that excessive growth of propionic-bacteria in Swiss cheese, especially in the later stages of ripening, tended to cause glass or checking.

Hammond and Reinbold (43) studied the split defect to determine whether it was brought about by either excessive carbon dioxide production or weakening of the body of the cheese by proteolysis.

Proteolysis was the most consistent index. Their study showed that there was some proteolysis, especially during the early stages of ripening, but the degree of proteolysis was not correlated with splitting. They found no obvious relation between splitting and carbon dioxide or volatile acid levels. Their data does not support the hypothesis that splitting is caused by high carbon dioxide pressure or by weakening of the body by proteolysis as measured by formal titration.

Park et al. (93) studied various factors that may be involved in the production of splits in Swiss cheese. Their results indicated that no correlation could be established between differences in salt distribution, pH, moisture level, and the extent of proteolysis in split and non-split cheese. They were able to show an increased incidence of splits, however, when propionibacterial strains, selected on the basis of growth at low temperature, were used in the manufacture of the cheese, but their results did not support the hypothesis that greater eye volume induced a greater tendency for cheese to split.

Effect of manufacturing procedures

Bolliger (9) investigated the effect of milk heat-treatment on the formation of eyes in Emmental cheese. He found that heating milk, at any temperature, caused a clear reduction in the extent of eye formation as compared to cheese made with raw milk. Burtscher (16) believed that pasteurizing the milk for Emmental cheese would be a means of simplifying the manufacture through controlling the

flora of microorganisms present. He determined that pasteurization had a favorable effect on formation of eyes but an unfavorable influence on flavor, texture, and keeping quality of the cheese.

Peters and Moore (97), in studying the effect of homogenization of milk used in the manufacture of Swiss cheese, determined that progressive increases in homogenization pressure reduced the quality of the ripened cheese. A high amount of pressure (2000 psi) caused a decrease in curd elasticity. With the use of this milk homogenization pressure, the cheese produced was criticized for defects such as blindness, glass, blow holes, and sponge. In a second report (98), Peters and Moore stated that to improve the effects of homogenization a smaller inoculum of propionibacteria should be used with milk homogenized at 2000 psi than that with unhomogenized milk.

Stettler (122) studied the effect of centrifugation and filtration of cheese milk on eye formation in 14 thousand Emmental cheeses. He found centrifugation had a significant beneficial effect on eye formation, for the experimental cheese was awarded, on an average, 1 extra point over the controls.

Some cheesemakers believe that the inoculation of too much propionibacteria will produce too many eyes. This is not necessarily true. Dorner et al. (23) found that the time and speed of eye formation depends upon the number of propionic-acid bacteria present in young cheese, but that no effect on quality was exhibited by increasing amounts over minimum number per cheese. Oehen and

associates (86, 88, 89) found that using varying amounts of propionibacteria for inoculation of cheese milk confirmed their belief that a surplus of culture did not effect cheese eye-quality adversely, but adding little or no propionibacteria was definitely detrimental to eye development in Emmental cheese. They found that even heavy overinoculation with propionibacteria did not cause any defects, but a weak inoculum usually resulted in cheese of inferior flavor and eye development.

Ystgaard et al. (146) presented a detailed account of their investigation into the effect of various factors used in the manufacture of a Swiss-type cheese made from pasteurized milk. They found the pH of fresh cheese was increased significantly by the addition of water to whey and by increasing the cooking temperature up to 43 C. The interaction between the water added to the whey, the cooking temperature, and the temperature in the curing room had a highly significant effect on the grades for eye formation. The best grades were obtained when a water addition of 5% was combined with a cooking temperature of 43 to 46 C and a curing temperature of 22 C, or a water addition of 15% was combined with a cooking temperature of 43 to 46 C and a curing temperature of 19 C. Combining the factors of large amounts of water added to the whey with low cooking temperature and low temperatures in the curing room proved extremely unfavorable to eye formation. The effect of the interaction between water added to the whey and the temperature in the curing room had a highly significant effect on

the occurrence of cracks in the body of the cheese. The occurrence of body cracks decreased with increasing water addition to the whey and with increasing curing temperature. The effect of water added to the whey was more pronounced at low than at high temperature levels in the curing room. Similar results were reported by Bijok and Domanska-Strzatkowska (8) when they determined that the addition of 20% water to the curd improved the eye formation and other characteristics of Emmental cheese.

Knĕz (65), in studying the size of curd on the numbers of eyes in Emmental cheese, found the number of eyes decreased with decreasing curd particle size. A somewhat conflicting report was given by Koestler (70), who determined that the quality of Emmental cheese was not significantly affected by the size of curd particles.

Oehen and Bolliger (87), in 1963, could not recommend the procedure of cold storage before ripening of Emmental cheese. They found that this procedure provided slightly improved eye formation but only at the cost of some injury to the characteristic texture and flavor. Later, in 1968, Kiermeier et al. (58) determined that holding fresh Emmental cheese below 10 C prior to ripening impedes propionic fermentation but leads to cheese of good flavor, characterized by the presence of well-formed, uniform eyes.

Effect of cheese constituents

Koestler (66) showed that a finely-harped curd tends to retain moisture and that the presence of small particles of cheese tends particularly to cause high moisture content leading to excessive

fermentation, oversetting, and generally defective ripening. Dorner and Sähli (25) expressed the belief that an insufficient rate and amount of drainage is a common cause of defects, and that the resulting high moisture content produces excessive acidity. This acidity prevents the formation of normal eyes and causes the curd to be firm and short so that cracks instead of eyes appear during the ripening. Sanders and associates (112, 113) found that a moisture level above 39.7% in "green" Swiss cheese was usually detrimental to cheese quality. This detrimental effect was most evident in eye formation. The defect known as "oversetting" was very pronounced in most of the high moisture cheeses. Sähli and Lehmann (111), in studying high quality Emmental cheese, found an average moisture content of 36.9% at 3 months and 34.5% at 6 months of age. These values were established to obtain some index for high quality Emmental cheese. There is general disagreement as to the desired moisture level and a large number of figures have been reported (113). Sanders et al. (113) studied the relationship of moisture in Swiss cheese to quality and yield, and determined that a moisture content above 39.7% was generally detrimental to the quality of the cheese. Their study presented effects of numerous variables in the milk and make process upon the moisture content of the cheese. They found that cheese made from milk which had been previously ripened with a lactic culture showed a relatively rapid development of acidity on the press after dipping. These ripened-milk cheeses in which the acid was produced

rapidly contained some glass (splits) and were slightly short and inelastic in texture and inferior in quality.

One of the real difficulties encountered in the manufacture of rindless block Swiss cheese is with brining. It is necessary to obtain a certain level of salt in cured cheese to compliment its flavor. Olšanský et al. (90) studied the effect of salt content on the quality of Emmental cheese and found that a cheese with 1.0% salt produced the best quality. With increasing salt content, the firmness, elasticity, and viscosity increased to produce undesirable cheese. Babel and Hammer (6) reported that a delay in salting of the cheese affected the salt content and growth of the propionibacteria. They found that a 2-day delay in salting resulted in a slightly sweeter flavor, slightly larger numbers of propionibacteria, and eyes extending nearer the surface than with the normal immediate salting procedure. Most propionibacteria do not readily grow in more than 3% salt in moisture. The normal salt concentration in wheel Swiss cheese 7/8 inch from the edge may be from 1.8 to 3.6% (103), thus forming the firm rind. Uotila (130) studied the differences in the contents of dry matter, fat, and sodium chloride of different parts of Finnish Emmental cheese and assumed that the gläsler (split) defect was associated with variations in the contents of dry matter and NaCl in different layers of cheese.

It has been suggested that the late fermentation and continued growth of the organisms could be attributed to the persistence of

slight amounts of sugars present in the cheese (52). Glucose disappears very rapidly, then the lactose, but galactose may persist for as long as 72 hr (11). Ritter et al. (107) found only residual galactose and no glucose and lactose in 24- to 48-hr old Emmental cheese.

Effect of physical and chemical factors in cheese

Koestler (67) determined the importance of hydrogen ion concentration on certain properties of hard cheeses. The pH of 3-month old Emmental cheese ranged from 5.45 to 5.75. Gruyère cheese was higher by approximately 0.1 pH unit. By taking into consideration the fact that normal ripening causes an increase in pH, Koestler believed that Emmental cheese 4-days old should have a pH of 5.10 to 5.40. Frazier et al. (33) noted that Swiss cheese showed a fairly rapid drop in pH during the first 7 hr in the press and a more gradual decrease thereafter. Acid production that was too rapid in the press resulted in rapid drainage. Slow acid production favored insufficient drainage and delayed eye formation. Burkey et al. (15) found large differences in pH between the interior (pH 5.9-6.1) and the portion just beneath the rind (pH 5.6-5.85) of the cheese. This condition resulted in insufficient drainage and high moisture content. The difference in pH was thought to cause such defects as checking and glass near the rind. On the other hand, Andres and Bolliger (2) ascertained that no greater than 0.1 pH unit 48 hr after manufacture was found in different parts of the cheese. Doležálek et al. (22) determined

that the propionic-acid fermentation and eye size of Emmental-type cheese appeared to be more affected by the initial pH after pressing than by the size of the inoculum of the propionibacterial culture. Many varied results have been published as to the desirable pH level during curing periods that would ultimately effect eye development, flavor, and the occurrence of defects (24, 34, 60, 74, 107, 111, 137).

Koestler (68), in 1940, attempted to more precisely define the elasticity of Emmental cheese and relate it to other qualities of a cheese slurry. He proceeded on the supposition that the elastic qualities of the cheese mass were essentially derived from the fundamental constituent, calcium phosphocaseinate. He concluded that elasticity is strongly temperature variable, is affected by pressure, and that no relationship appears between the salt and water contents normally found in the cheese. Later, Koestler (69) found that neither the range nor the degree of protein decomposition appear to have any effect on texture, which varies not only from cheese to cheese but, also, within an individual cheese. He reported that lactate formation plays a decisive role in promoting fine texture. Klimouskii et al. (64) investigated the role of propionibacteria in the proteolysis of Soviet Swiss-type cheese and found that they did not significantly affect proteolysis. Reports have been published concerning the inability to correlate proteolysis to texture and elasticity (57, 124, 139) and proteolysis to the occurrence of the split defect

(43, 93).

Effect of packaging films and wrappers

The manufacture of Iowa Swiss-type cheese was published some 30 years ago and since that time various modifications in method of manufacture have taken place (19). In recent years emphasis has been placed on the manufacture of cheese in the form of a rindless block that can be cut into small portions, packaged, and marketed with a minimum of waste (19).

The rind on Swiss cheese serves as an excellent barrier for carbon dioxide diffusion. Upon cutting for sale, the rind is removed and discarded, thus causing an economic loss to the manufacturer. With the development of rindless blocks considerable savings were derived when the entire cheese could be utilized for sale.

Since the rind serves as an excellent barrier for carbon dioxide diffusion, studies have been performed to evaluate the effect of various coverings on diffusion in the rindless block with comparisons to the conventional Emmental or Swiss cheese wheel. Burkhalter et al. (13) treated the surface of cheese by various means to determine the effect on quality. They found that by using artificial resin dispersions they could produce cheese of body, flavor, and eye formation comparable to conventional control rind-formed cheese. Burkhalter and Obrist (14) reported that Foodplast (a plastic-like synthetic covering) coated on the cheese was beneficial in saving labor and reduced cheese weight losses

when stored at low humidity. Under high humidity conditions mold growth was impossible to control. Futschik (38) determined that cheese wrapped in highly impermeable films provided unsatisfactory results when discoloration and liquefaction occurred. With films of greater permeability satisfactory appearance, flavor, texture, and eye formation of the resultant cheese compared well with those obtained by normal ripening or paraffining methods. Swartling (125) found that when fresh Herrgård and Svecia cheese were wrapped in a highly impermeable film, the gasses evolved were unable to escape and the package became distended. Flückiger and Roy (31) studied various types of plastic wrappers on Emmental cheese to determine the most effective type to prevent the ingress of oxygen and still preserve flavor. On storage, the carbon dioxide content increased in impervious packages, but declined with those that were oxygen permeable. Mold formation on the cheese was the problem in the high oxygen transmittability wrappers. Dürichen et al. (26) attempted to correlate gas concentration changes in Emmental cheese packaged in plastic films to the films significance in retention of cheese quality. They found that a 100% carbon dioxide rather than a nitrogen atmosphere in storage resulted in optimal retention of cheese quality. Ziegler (147) studied the factors affecting choice of film, shape, and size of Emmental cheese to be packaged and modifications in the manufacturing techniques, packaging and storage. Cheese packaged in films were considered to be rindless and these cheeses had a satisfactory

odor, flavor, and plasticity.

To determine if the carbon dioxide permeability of the rind is related to storage durability, Kiuru and Uotila (62) studied various methods to render rinds more permeable to carbon dioxide. They found that when the rind was made more easily permeable to carbon dioxide by covering the cheese with a plastic resin emulsion the cheese was less susceptable to swelling and glasler (split) defect than cheese with a rind of poor carbon dioxide permeability. They determined that the gas permeability of the rind in normal Emmental cheese decreased when the surfaces dry out and the incidence of the split defect dramatically increased.

The more impermeable the wrapper, the less rind and mold formation will occur. This is highly desirable except that the more impermeable the wrapper (or the thicker and denser the rind in conventional wheel Swiss cheese), the greater will be the danger of splits. Until a film is developed that will let CO_2 out and not permit the entrance of oxygen, a compromise is necessary (102).

Growth and Carbon Dioxide Production of Propionibacteria

Propionibacteria may be characterized, in general, as being Gram-positive, catalase-positive, nonsporeforming, nonmotile, facultative anaerobic, lactate fermenting, rod-shaped bacteria. They are considered to be heat resistant and able to survive pasteurization temperatures (16, 126). Eleven species of

propionibacteria are presently recognized; their complete characterization can be found in reviews by van Niel (133) and in Bergey's Manual of Determinative Bacteriology (10). These organisms play important roles in several industrial processes. For instance, they are important in the development of the characteristic flavor and eye production in Swiss-type cheeses; they produce as a by-product of their fermentation large quantities of propionic acid, and they synthesize large amounts of vitamin B_{12}.

Substrate and product inhibition

Growth and fermentation of propionibacteria are stimulated by a variety of compounds utilized in their nutrition. It, therefore, is likely that they can be inhibited by other compounds also involved in their metabolism. For example, it has been shown that growth of five strains of propionibacteria was inhibited to varying degrees in media containing up to 4% calcium or sodium propionate (4); the sodium salt was more inhibitory than the calcium. Lactate (calcium and sodium salts) also inhibited growth of propionibacteria (5); inhibition depended upon the growth medium rather than upon the presence of undissociated acid. According to Antila (3), acetate can stimulate growth of propionibacteria; at relatively small concentrations acetate, however, could suppress growth.

Recently, Neronova and associates (82, 83) investigated the growth inhibitory effect of the fermentation products of propionibacteria. They reported that propionic acid suppressed growth less

than acetic acid and that the magnitude of over-all inhibition was not additive when both acetic and propionic acids were present. When the two acids were present in the medium, the one at the highest relative concentration was the growth-controlling factor. By using lactate as the primary nutrient source, the concentration and conditions at which the substrate was inhibitory also were determined.

Since sodium chloride is used in the making and curing of Swiss cheese varieties, it is important to understand what effect it has upon the growth and metabolism of propionibacteria. According to Peltola (96), sodium lactate is fermented in a nutrient solution containing 4% salt. The beginning of gas formation was retarded by higher additions of salt. Rollman and Sjöström (109) experimented with different levels of salt ranging from 0 to 8% in a lactate substrate. They found that, with a fast growing strain of propionibacteria, concentrations of 6% salt were required to impede growth at pH 7.0, and 3% at pH 5.2; whereas, a slow growing strain had greater salt tolerance at pH 5.2 than at 7.0.

Vedamuthu et al. (135) have demonstrated the presence of an inhibitory factor in milk serum and cheese whey for 22 of 56 strains of propionibacteria. This nondialyzable fraction, subsequently shown to be a globulin fraction, was quite active against some strains of propionibacteria. Large inocula of propionibacteria reversed the inhibition. Other reasons for insufficient growth of

propionibacteria have been given by Kurmann (73), such as too high cook temperature, copper contamination, manufacturing procedure short cuts, and/or antibiotics.

Associative action of other microorganisms

The growth and fermentative ability of propionibacteria can be stimulated or inhibited by associative growth of other microorganisms. As early as 1921, Sherman and Shaw (118, 119) and Whittier and Sherman (140) made extensive studies of the stimulating effect of associative growth of bacteria on the propionic-acid fermentation. They found that propionibacterial fermentations were stimulated during interaction with different types and species of bacteria, some of which were incapable of fermenting the available carbohydrate. They did not offer any explanation for this phenomenon. Similar observations were made by von Freudenreich and Orla-Jensen (138). Several conflicting reports have since appeared concerning the stimulatory-inhibitory role of the lactobacilli on propionic-acid bacterial growth and fermentation. Kuiru (60) found that a strain of Lactobacillus lactis had an appreciable delaying effect on the fermentation reactions of Propionibacterium peterssonii. Winkler (142) tested 12 strains of lactobacilli and found 2 strains of Lactobacillus lactis, 2 of Lactobacillus helveticus, and 1 of Lactobacillus acidophilus inhibited the growth of certain strains of propionibacteria. He determined that two inhibitory agents, one heat-labile and the other heat-stable, were involved. Recently, Nieuwenhof et al. (84) reported data supporting

the contention that lactobacilli stimulate growth of propionibacteria. They offered no explanation for the stimulating effect but discounted the possibility that variations in the pH of the cheese were responsible. Hietaranta and Antila (53) found cell-free, chalk-milk culture-filtrates of L. helveticus stimulatory, and Hunter and Frazier (56) determined that L. helveticus produced metabolic products with stimulating activity. These metabolic products could contain excreted biotin and pantothenate, which have been proven stimulatory. Hunter (55) studied the gas-producing abilities of several genera of associated Swiss cheese bacteria. He determined that P. shermanii produced significantly more gas in skimmilk cultures previously fermented by lactobacilli as compared to skimmilk fermented by streptococci cultures. He also tested the stimulatory effect of dialysates of disrupted lactic cultures and found the dialysates of several strains to be definitely stimulatory to gas production. Klimovskii and Alekseeva (63) determined that the metabolic products of several lactobacilli were stimulatory to the growth of propionibacteria.

Ritter et al. (106) investigated the stimulatory and inhibitory effects of micrococci on propionibacteria. In their study, relative quantitative differences in the volatile acids produced by a propionibacterial test strain in pure culture, and in association with micrococci in the test medium, were used to measure stimulation or inhibition. When the micrococci were present, increased amounts of volatile fatty acids were produced by the

propionibacteria. Ritter and Schwab (105), in a later report, noted that the presence of micrococci caused approximately a 20% increase in carbon dioxide production by propionibacteria in cheese.

Winkler (141), studied the antibiotic action of other microorganisms and found definite inhibition of growth of the propionic-acid bacteria. He determined that certain strains of Pseudomonas aeruginosa were highly inhibitory to the growth of propionibacteria. Strains of Bacillus subtilis, Bacillus mesentericus, Bacillus mycoides, and Staphylococcus spp. were inhibitory to a lesser degree.

Physical factors affecting growth

Many investigators have utilized 30 C, considered to be the optimum growth temperature, for culturing propionibacteria (10). Orla-Jensen (92) stated that propionibacteria grow at temperatures between 15 and 40 C, and Kurman (72) found that the best cheese-plant storage temperatures for cultures range between 5 and 15 C when held 1 month between transfers. Since it was generally believed that propionibacteria are able to grow at temperatures not lower than 10 to 15 C, Park et al. (94) set out to determine their minimum growth temperature. They found that the assumed lower limit of 10 C was incorrect and that propionibacteria will grow at temperatures between 2.8 and 7.2 C during a 4-month incubation period. Such low-temperature growth patterns have a special significance in the manufacture of Swiss-type cheeses.

When growth of various strains of propionibacteria associated with Swiss-type cheeses occur at low temperatures in this range, defects such as splits may result (52).

In studying the influence of hydrogen-ion concentration upon the growth of propionibacteria, Tittsler (127) found that the optimum pH was 6.5 to 7.0. Whittier and Sherman (140) studied numerous factors affecting the propionic-acid fermentation. The relation of hydrogen-ion concentration to the rate of propionic-acid fermentation distinctly showed that the optimum level is pH 7.0 and that at pH 5.0 there is practically no growth and consequently, little production of propionic acid. Later, Tittsler and Sanders (128) reported that, with the decrease in pH from 6.0 to the "critical point" (pH 5.0), initiation of growth was increasingly delayed and rate of growth was reduced. The bacteria lost viability at pH values below the critical level for their growth. Kurtz et al. (74) investigated the interrelationships between pH and population levels in Swiss cheese and determined that an upward increase in pH from 5.0 generally correlated with increases in populations of P. shermanii in the cheese. Kiuru (60) followed the propionic-acid fermentation in lactate milk by measuring carbon dioxide evolved. He found the fermentation optimum of the propionibacteria lies at pH 6.0 with the minimum at pH 5.0.

The carbon source plays an important role in the nutrition of propionibacteria. Antila (3) found that the formation of lactic

acid during cheesemaking provides a suitable fermentable substrate for propionibacteria. He determined that these bacteria will best ferment lactic acid in the presence of yeast extract. Antila and Hietaranta (5), however, stated that, depending upon the nutrient medium present, a heavy lactate concentration hinders growth and fermentation. Hietaranta and Antila (53) determined yeast extract to be definitely stimulatory, but the best stimulation came from cell-free filtrates of milk cultures of Streptococcus thermophilus and Lactobacillus spp.

Emilsson and Sjöström (27) stated that propionibacteria cannot ferment the citric acid in cheese, but, according to Antila (3), this observation was probably made with a strain that grew poorly in cheese. Antila (3) found that citrate decomposition will occur in cheese if lactate is present. Hietaranta and Antila (53) proved that propionibacteria are able to break down citrate.

Kiuru (61) established that metallic iron and iron ions stimulate the production of propionic acid, and, according to Pulay et al. (100), P. shermanii requires a minimum amount of iron for the production of carbon dioxide. For additional information concerning the growth of propionibacteria, a detailed account is provided in a review to be published by Hettinga and Reinbold (48).

Effect of copper on growth

Mueller et al. (81) presented data which demonstrated that bacteria associated with Swiss cheese manufacture vary considerably in their behavior toward copper. Copper increased the lag phase

and decreased the growth rate of three P. shermanii cultures tested. A similar lag in volatile acid and carbon dioxide production was observed. Preliminary evidence indicated that high levels of copper (18 ppm) in Swiss cheese exerted an unfavorable influence on the course of ripening, whereas a normal and satisfactory ripening occurred in cheeses containing 0 to 0.5 and 7.5 to 8.0 ppm copper. Rough eyes and splits, however, appeared under the surface of the experimental cheese. Burkhalter (12) stated that the use of stainless steel vats for the manufacture of Emmental cheese could not be recommended due to adverse effects on the fermentation, ripening, and cheese quality. Flückiger and Zürcher (32) determined that the use of stainless steel vats instead of copper vats in the manufacture of Emmental cheese tended to produce an increase in volatile acids and carbon dioxide production, thereby indicating that copper had a deleterious effect on the growth of the propionic-acid bacteria. The disposition for splits was not greater than in cheese made in copper vats. Kurmann (73), on the other hand, reported on problems Gruyère factories were having with poor eye formation in the cheese caused by insufficient propionic fermentation. Several possible reasons were given, including an excessive increase in copper content of milk from overnight storage in copper kettles. Maurer (79) made an extensive study of the effect of copper on the growth, and carbon dioxide production of propionibacteria in broth cultures and in Swiss cheese. He determined that a copper concentration of 16 ppm severely inhibited

the growth and carbon dioxide production of the two P. shermanii strains and of the single P. arabinosum strain tested in broth cultures. Swiss cheese containing 11 ppm copper required more time to develop eyes. The velocity of gas production was tested in two lots of cheese manufactured with two different P. shermanii strains and was found to be markedly reduced as compared to the two lots of negative controls. Several other reports (59, 115, 148) substantiate the deleterious effects copper may have on the growth and fermentative abilities of the propionibacteria, especially when present in higher concentrations.

Growth in cheese

Sherman (117) found that three different cultures could be used to control fermentations in Emmental cheese: Lactobacillus bulgaricus to suppress gassy fermentation produced by undesirable bacteria, Bacterium acidi propionici to insure development of the characteristic flavor as well as formation of eyes, and Lactobacillus casei to control overswelling. The growth and activity in the cheese kettle of a series of cultures consisting of Streptococcus thermophilus, L. casei, L. bulgaricus, and P. shermanii were investigated by Frazier et al. (35). S. thermophilus increased in numbers during the kettle process, and the increase was most rapid when the temperature of the kettle contents was 53 C. Lactobacillus casei, L. bulgaricus, and P. shermanii did not increase in numbers during the kettle process but usually decreased. Streptococcus lactis usually increased in numbers

during the first part of the kettle process but was stopped by the cooking temperature. Frazier et al. (33) also investigated the growth of various organisms during the time the cheese was in the press. They found that the lactobacilli initiated growth usually after the 5th hr of pressing. The P. shermanii did not grow.

Demeter et al. (21) determined the growth of the propionic-acid bacteria and found their growth to be opposite to that of the lactic-acid bacteria. The propionibacteria reach their lowest level during brining and then, during the hot room period, when the formation of eyes takes place, they increase in numbers so immensely that they supersede all other kinds of bacteria. Oehen et al. (89) found the increase in numbers of propionibacteria in the ripening of Emmental cheese was paralleled by increases in the volatile fatty acids and CO_2 production. Reinbold et al. (103) in studying the distribution of propionibacteria in Swiss cheese determined that the numbers of propionibacteria increased, though not proportionally, from the exterior to the interior of the cheese. Their data suggested that the decrease in numbers toward the exterior was probably the result of such unfavorable conditions as lower moisture, higher salt concentration, and higher oxidation-reduction potential.

Metabolism and enzyme reactions

Fitz (28), in 1878, began what is considered today as the first work of importance on the biochemistry of the propionibacteria. Study of the propionibacteria was completely overlooked until von Freudenreich and Orla-Jensen investigated other aspects of the propionic-acid fermentation. Orla-Jensen (91) first conducted research concerning the formation of eyes in Emmental cheese and, later, von Freudenreich and Orla-Jensen (138) were able to isolate pure cultures and to report the first extensive description of the propionic-acid organisms. Most of the attention to these organisms was directed to their effects in Emmental cheese and their taxonomy, until Virtanen (136), van Niel (133), and, later, Wood and Werkman (145) extensively investigated their fermentative abilities.

Van Niel (133) has made probably the most comprehensive contribution to our knowledge of this genus. His dissertation contains a full discussion of the characteristics of the species and a key for their differentiation. Methods of isolation also are discussed fully as well as a comprehensive study of the biochemistry of the propionibacteria. Quantitative studies on the fermentation of lactic and pyruvic acids, glucose, glycerol, and starch enabled van Niel to draw conclusions as to the mechanism of the propionic-acid fermentation.

For a more complete review of the early literature concerning the production of propionic acid by propionibacteria, reference should be made to the work of van Niel (133) and that of Wood (144).

Wood and his associates have performed the majority of the research of fermentation by propionibacteria. A complete review by Hettinga and Reinbold (49, 50) will be published on the metabolism of propionibacteria.

The formation of propionate by propionibacteria has been extensively investigated, and the pathway is known to involve a number of enzymes that have been purified and studied. These enzymes catalyze a wide variety of biochemical reactions found in propionibacteria, and a complete review of these enzymes and their reaction mechanisms also will be published (49).

Lactic dehydrogenase is one of a large number of enzymes in the group of dehydrogenases that are linked to the coenzyme NAD. It specifically catalyzes the reversible reaction:

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \longrightarrow \text{lactate} + \text{NAD}^+$$

The overall equilibrium of this reaction favors the forward reaction because of the large negative value of the standard free energy (ΔG^o + -6 kcal) (75). This reaction occurs as part of glycolysis in the majority of all living cells and NAD-linked lactic dehydrogenases are found through the biosphere. Lactic dehydrogenase has been isolated from a large variety of sources and appears to be quite uniform in most of its properties regardless of the biological origin. It has a molecular weight of 140,000, is specific for L-lactate, and shows a preference for NAD over NADP as the coenzyme (143). The occurrence in P.

pentosaceum of enzymes able to catalyze an oxidation of lactate in the presence of fumarate has been demonstrated by Barker and Lipmann (7). The evidence presented by these authors suggests the participation in the system of a type of lactate dehydrogenase different from that found in animal tissues. Later, Molinari and Lara (80) characterized the properties of this enzyme in P. pentosaceum and found them similar to lactate dehydrogenases from other sources.

The oxidation of pyruvate to acetyl CoA, catalyzed by the pyruvate dehydrogenase system, is in fact a very complex process, which has been studied in detail. The overall equation is:

$$\begin{array}{l} \text{Pyruvate} + \text{NAD}^+ \\ \quad + \text{CoA-SH} \end{array} \longrightarrow \begin{array}{l} \text{acetyl-S-CoA} + \text{NADH} \\ \quad + \text{H}^+ + \text{CO}_2 \end{array}$$

Because of the large decrease in the standard free energy (ΔG^o + -8.0 kcal), this reaction is essentially irreversible (75). This reaction is considered to be a complex that catalyzes the total sequence and involves three enzymes: pyruvate decarboxylase, with thiamin pyrophosphate as coenzyme; lipoyl reductase-transacetylase with two coenzymes, lipoic acid and coenzyme A; and dihydrolipoyl dehydrogenase, with two coenzymes, FAD and NAD. A complex of these enzymes has been isolated from different sources (143). Allen et al. (1) briefly describes the occurrence of pyruvate dehydrogenase in P. shermanii.

The NAD-linked L-malate dehydrogenase catalyzes the oxidation

of L-malate to oxaloacetate as follows:

$$\text{L-malate} + NAD^+ \longrightarrow \text{oxaloacetate} + NADH + H^+$$

Although the reaction is endergonic as written, it goes in the forward direction very readily in the cell because of the rapid removal of the reaction products oxaloacetate and NADH in subsequent steps. $NADP^+$ is only feebly reduced by the enzyme (75). Allen et al. (1) purified and characterized this enzyme in P. shermanii. They were able to obtain highly active purified preparations of this enzyme which compared favorably in specific activity to malate dehydrogenases from animal sources.

EXPERIMENTAL MATERIALS AND METHODS

Source of Milk

Grade A milk, obtained from the Iowa State University Creamery, was used in this investigation in the manufacture of Iowa-type Swiss cheese.

Manufacture of Iowa-Type Swiss Cheese

The method of manufacture of Iowa-type Swiss cheese was first published some 30 years ago (42). Since that time various modifications in the method of manufacture have been reported (19, 102), and the following is a simplified procedure for the manufacture of rindless block Iowa-type Swiss cheese.

Milk was pasteurized at 71.7 C for 16 sec, standardized to 2.8% milkfat, placed in a 2270-kg cheese vat and adjusted to 32 C. An active lactic culture, Streptococcus thermophilus, Lactobacillus bulgaricus, and a strain of a particular Propionibacterium spp. were added to the milk at the following rates of inoculation: 0.1%, 0.25%, 0.005%, and 0.016%, respectively. The milk was allowed to ripen for 30 min to develop 0.005 to 0.01% titratable acidity. After dilution with 40 volumes of cold water, the rennet was mixed with the milk at the rate of 75 ml/454 kg of milk. Agitation in the vat was stopped within 2 min and the currents in the vat were arrested to allow the milk to become quiescent before coagulation. When the curd broke clean, 25 to 30 min after setting, it was first

cut lengthwise of the vat with the ¼-inch horizontal knife, then lengthwise and crosswise with the ¼-inch vertical knife. A stirring paddle was replaced by a ¼-inch curd knife and agitation was continued for 5 min at high speed. When the coagulum had been uniformly reduced in size to cubes of about 3/8 to 1/4 inch, the mechanical harp was replaced with the original stirring paddle and the curd was agitated at high speed for an additional 10 min. Agitation was stopped and while the curd settled, one-third of the whey, measured accurately with a depth gauge, was rapidly drained. Water at 62.8 C, equal to the volume of the whey removed, was added in three portions at 10-min intervals with continuous stirring, to raise the temperature of the vat contents to 41 C. The curd was stirred continuously in the diluted whey for 105 to 110 min. The stirring time was decreased or increased depending upon the amount of moisture desired in the curd. The curd was allowed to settle, a cheese cloth covered dam was placed against one interior wall, and perforated boards were placed on each end of the curd to facilitate drainage and to provide the proper size and shape to the cheese blocks to be formed (the proper distance being that space that will give evenly shaped blocks which will fit into 20-lb Wilson hoops without curd loss). The curd mass was gently leveled, followed by placing a press plate over the submerged curd and cheese cloth, and weight (600-800 lb) was added to aid the matting of the curd into a solid mass. The cheese curd mass was allowed to press under whey for 30 min. The remaining whey was then drawn and pressing was continued

for an additional 30 min. The weights, press board, and cheese cloth were removed and the curd mass was cut into measured blocks of approximately 22 lb each. The blocks of cheese were removed and placed in metal hoops, lined with parchment press papers or cloths. The cheeses in the hoops were pressed overnight in a conventional horizontal press at a total pressure of 1500 lb. The pressure was applied slowly and after 1 hr the cheese was checked to determine if the press cloths were tightly against the cheese in the hoops. The next day the cheeses were removed from the hoops and press cloths, and placed in a saturated brine solution at 7 C for 48 hr with one turning after 24 hr. The cheeses were dried for 3 to 4 hr at 7 C, wrapped in a low moisture impermeable film, over-wrapped with Kraft paper, and placed in expandable wooden boxes with rubber bands secured around each box. In several experiments the cheese blocks were wrapped in various films of different oxygen and carbon dioxide permeability. The wrapped cheese remained in the cold room at 7 C for a total of 10 days from the date of manufacture and was then transferred to the hot room at 21 C to allow eye development. Duration of the hot room treatment depended upon the rapidity at which the proper eye size developed in the cheese. Close observation of the cheese by periodic withdrawal of a sample with a cheese trier from several blocks was necessary to determine proper eye formation. When sufficient eye formation had developed (usually about 3 weeks), the cheese was removed from the hot room and placed in a finish cooler

at 4 C for final curing.

Determination of Titratable Acidity of Milk and Whey

Titratable acidity of milk or whey was determined by titrating a 9-ml sample with 0.1 N NaOH to the first persistent pink color using 10 drops of phenolphthalein indicator (1.0% in 95% ethyl alcohol).

Sampling Cheese for Analysis

Samples of cheese for analysis were taken with a cheese trier half-way between the center and outer edge of the block of cheese. The samples were transferred to Whirl-pak bags (Nasco, Fort Atkinson, Wisconsin), sealed, and placed at -20 C for storage until analysis.

Proteolysis by Dye Binding in Cheese

An index of the extent of proteolysis was determined by measuring the total protein by the dye-binding technique of Hammond et al. (44), using orange G (Matheson, Coleman, and Bell, Matheson Co., Norwood, Ohio). The dye solution (1 mg/ml) was prepared by weighing 1.0638 g orange G (assayed 94% dye, dried for 3 hr) and 0.3020 g oxalic acid into a 1000-ml volumetric flask and diluting to volume with distilled water.

One hundred and fifty mg of ground cheese were weighed into a 15 x 2 cm test tube, stoppered, and frozen until the test could be

completed. At that time, 15 ml of dye solution were added with a volumetric pipette. The sample was then homogenized in a specially designed test tube homogenizer for 1 min. The tubes and their contents were held overnight and then centrifuged at 2500 rpm for 15 min in a clinical centrifuge. Absorbance was read in a Beckman model DU spectrophotometer (Beckman Instruments, Inc., Fullerton, California) using a "flo-thru" curvette at 475 nm against a blank. The blank was prepared for the entire experiment by diluting the original dye solution with an equal volume of distilled water. Results are expressed as units absorbancy per g cheese.

Determination of Lactic Acid in Cheese

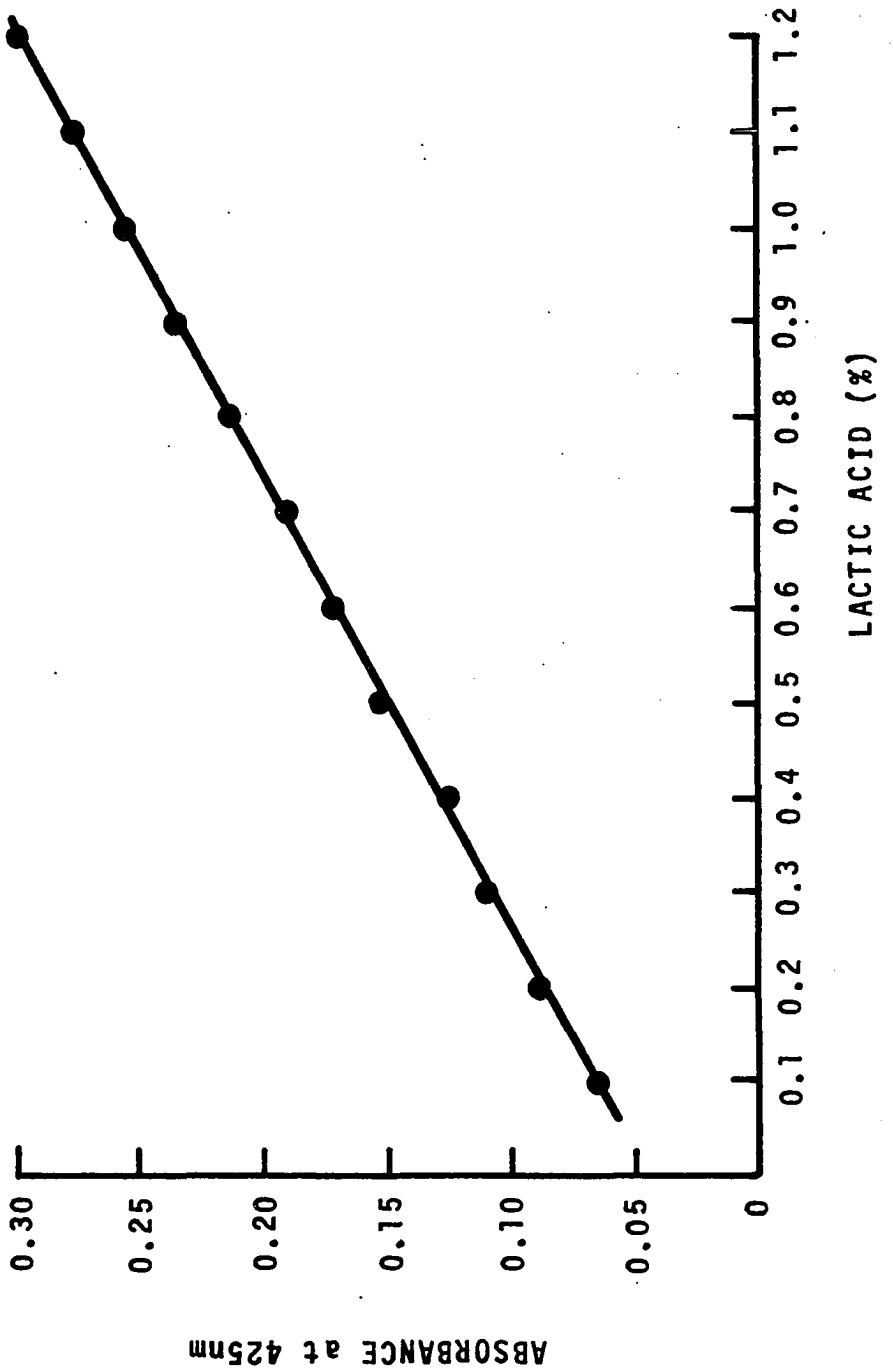
A modification of the methods of Harper and Randolph (46) and Ling (76) for the determination of lactic acid in Swiss cheese was used. Ten grams of cheese were blended with 90 ml of distilled water until homogeneous, then diluted with distilled water 1:10. Twenty-five ml of representative sample were measured into a 125-ml Erlenmeyer flask; 10 ml of barium chloride reagent (98.8 g $BaCl_2 \cdot 2H_2O$/1000 ml H_2O) were added and mixed thoroughly. While swirling, 5 ml of 0.66 N sodium hydroxide and 5 ml of zinc sulfate reagent (225 g $ZnSO_4 \cdot 7H_2O$/1000 ml H_2O) were added, the mixture shaken vigorously, and then filtered through Whatman no. 40 filter paper. A reagent blank was prepared by the same procedure, except that 25 ml of distilled water were substituted for the sample. Color was developed from the lactic acid in the clear filtrate by

pipetting 10 ml into a clean dry test tube, and adding 1 ml of freshly prepared ferric chloride solution (5 g $FeCl_3 \cdot 6H_2O$ dissolved in 12.5 ml 1.0 N HCl, made to 100 ml with distilled water and diluted 1:4 with distilled water as required), and transferred to optically matched cuvettes. The transmittance of the sample was determined at 425 nm, using a Coleman model 11 spectrophotometer (Coleman Electric Co., Inc., Maywood, Ill.). The spectrophotometer was standardized by adjusting the reagent blank to 100% transmittance. The amount of lactic acid was determined from a standard curve prepared using lithium lactate (Figure 2). By this procedure the total lactate content of the cheese was determined and expressed as per cent lactic acid.

Estimation of Carbon Dioxide Production in Cheese

A method for the estimation of carbon dioxide released from wrapped Swiss cheese was developed for determining differences in carbon dioxide production between propionibacterial strains under normal cheese-curing conditions. Carbon dioxide production was estimated by placing approximately 46 kg of cheese in a molded polyethylene barrel and sealing the barrel to prevent leakage. The flow of air pumped from a simple aquarium pump was regulated at 1 cc/min by a Brooks-mite purge meter (Brooks Instrument Division, Emerson Electric Co., Hatfield, Pennsylvania). This air was bubbled through 1 N sodium hydroxide and a soda lime column to remove atmospheric carbon dioxide, through distilled water for

Figure 2. Relationship between absorbance and lactic acid concentration


ABSORBANCE at 425nm
0.30
0.25
0.20
0.15
0.10
0.05
0
0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2
LACTIC ACID (%)

rehydration, and into the air-tight barrel. The pressure produced within the barrel forced the air and the carbon dioxide released from the cheese through an outlet and into 100 ml of 1.0 N NaOH. The NaOH sample was protected by a water trap to prevent contamination by atmospheric carbon dioxide (Figure 3). Twenty-five milliliters of the 100-ml partially neutralized NaOH sample were pipetted and placed in a 100-ml beaker. Ten ml of 1 M barium chloride solution were added and the sample was titrated with 1.0 N hydrochloric acid to a phenolphthalein endpoint. Moles of carbon dioxide produced for a 24-hr period were calculated by the following equation:

$$\text{Moles } CO_2\text{/day/kg cheese} = (\text{meq NaOH/day/kg cheese})(\text{eq } CO_2)\ (1/1000)$$

Enumeration of Propionibacteria in Cheese

The pouch technique of Hettinga et al. (51) was used for the enumeration of propionibacteria.

Determination of Sugar in Cheese

The sensitive enzymatic method of Hettinga et al. (47) for the determination of glucose, galactose, and lactose in Cheddar cheese was used for determination of sugars in Swiss cheese. Figures 4 and 5 illustrate the relationship of glucose and of galactose concentrations to absorbance.

Figure 3. Flowsheet of the system employed for the measurement of carbon dioxide produced in Swiss cheese

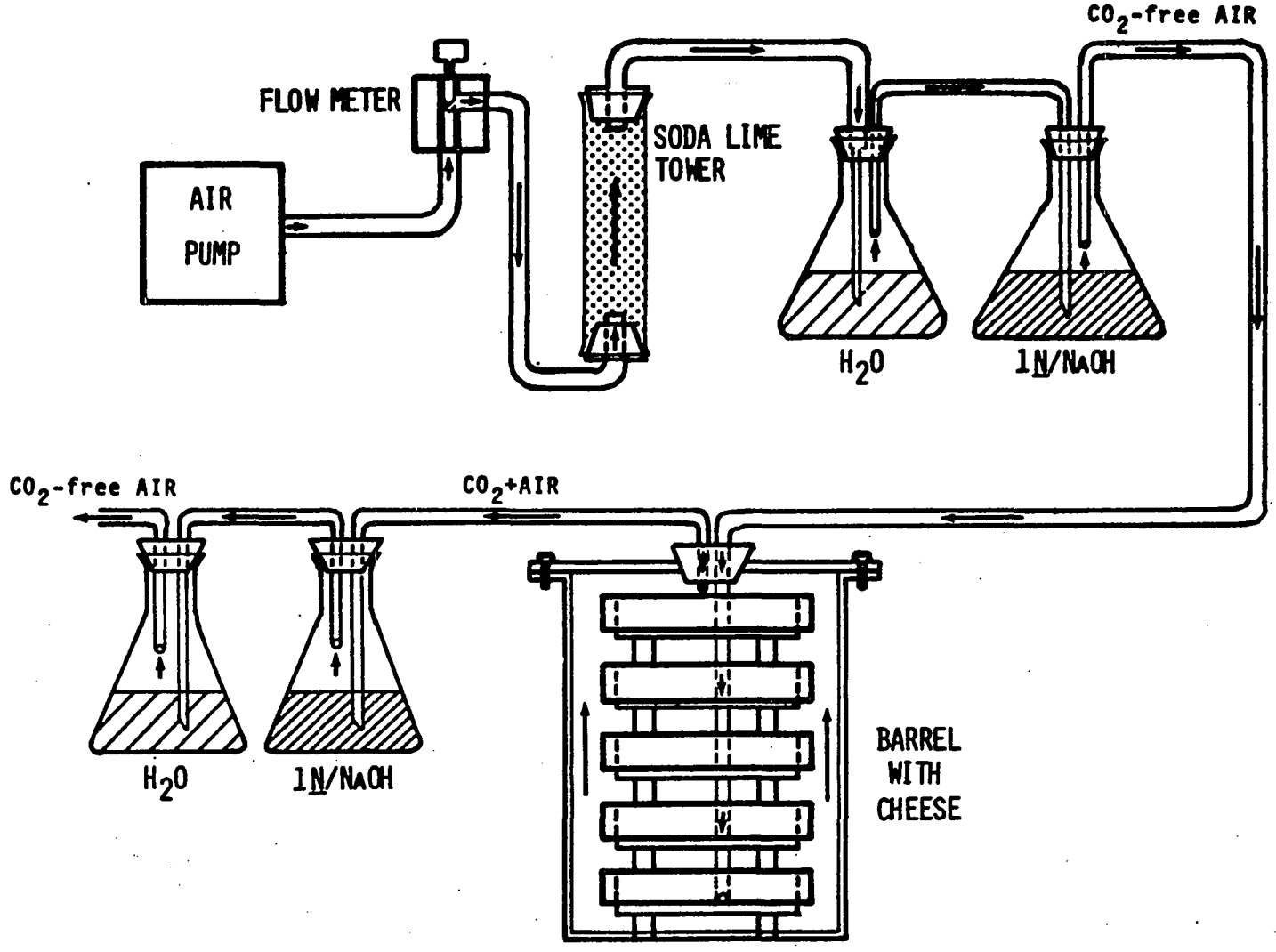

CO_2-free AIR
FLOW METER
AIR PUMP
SODA LIME TOWER
H_2O
1N/NaOH
CO_2-free AIR
CO_2+AIR
H_2O
1N/NaOH
BARREL WITH CHEESE

Figure 4. Relationship between absorbance and glucose concentration

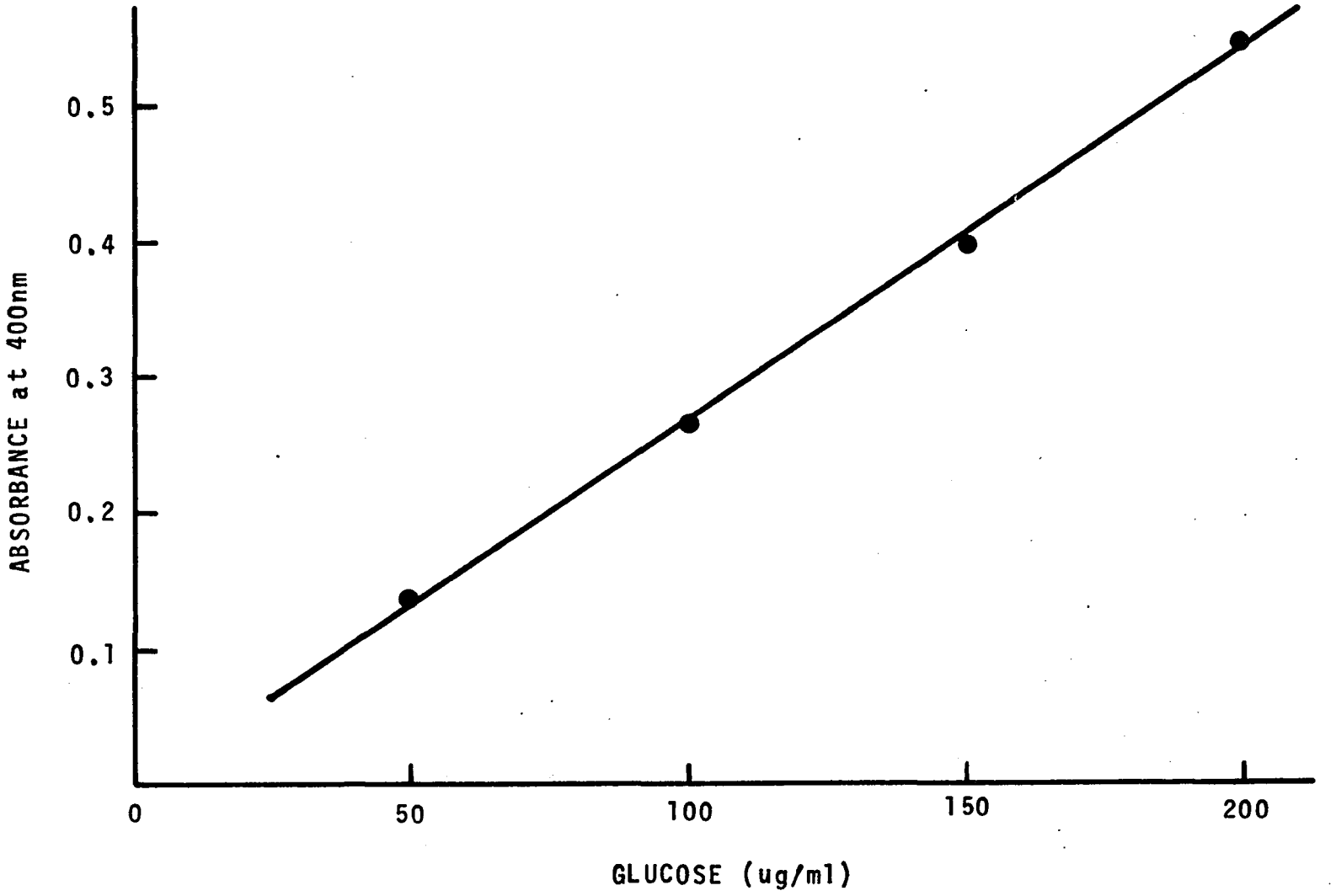
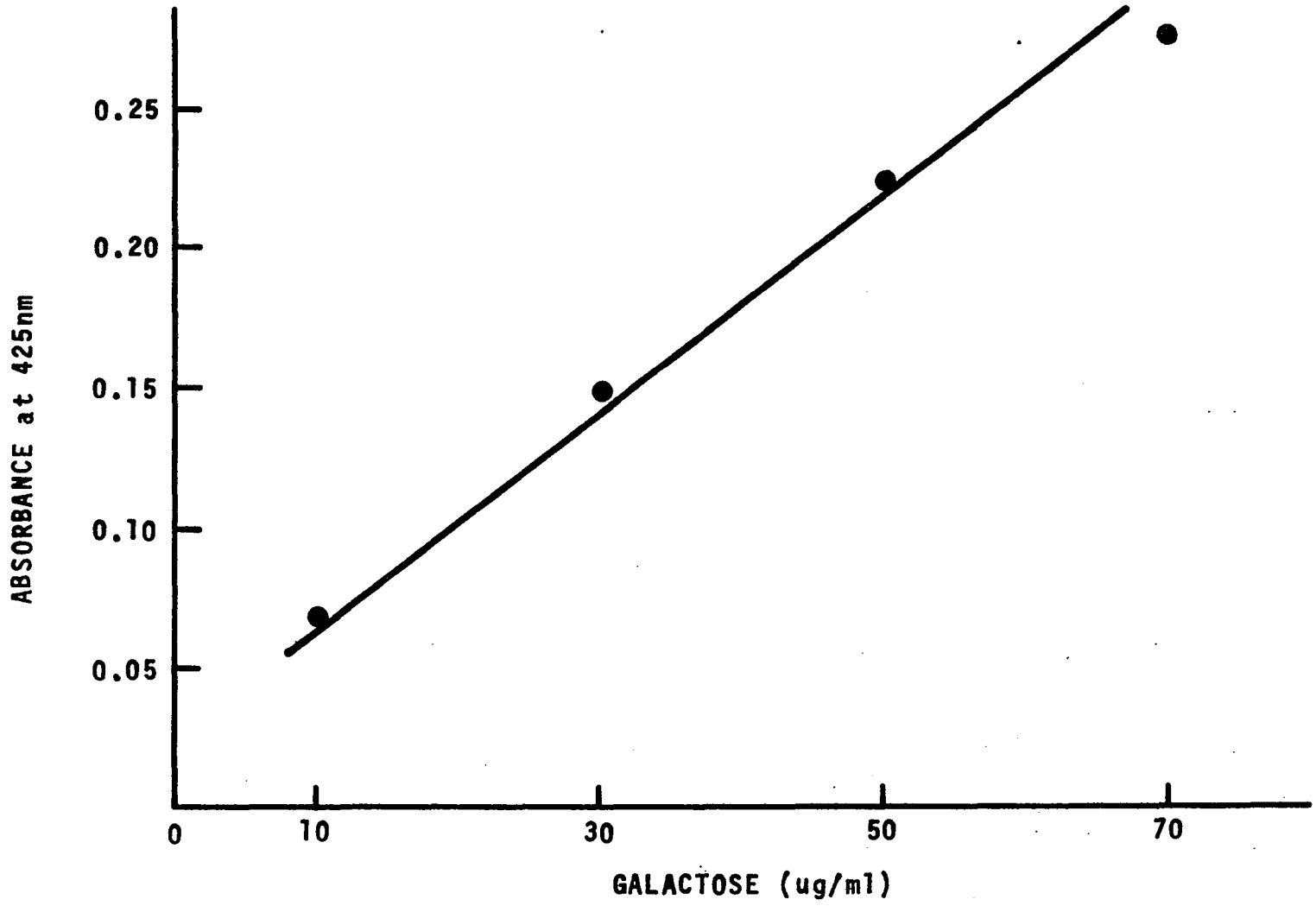

ABSORBANCE at 400nm
0.5
0.4
0.3
0.2
0.1
0
50
100
150
200
GLUCOSE (ug/ml)

Figure 5. Relationship between absorbance and galactose concentration


ABSORBANCE at 425nm
0.25
0.20
0.15
0.10
0.05
0
10
30
50
70
GALACTOSE (ug/ml)

Strains of Propionibacteria

Strains of propionibacteria were obtained from the culture collection of the Department of Food Technology, Iowa State University. The propionibacteria were originally obtained from the sources listed by Malik et al. (78) and as shown in Table 1.

Preparation of Cultures of Propionibacteria

All cultures were transferred daily on at least three consecutive days before being used. The cultures were purified by streaking on a Sodium lactate agar (51) plate and incubated using the Candle oats jar technique of Vedamuthu and Reinbold (134).

Determination of Gas Production by Propionibacteria

A modification of the methods of Parmelee (95) and Holmes et al. (54) were used to estimate gas production by propionibacteria. Sodium lactate broth (94) was filled to the base of the necks of 50% cream-test Babcock bottles and was inoculated with various strains of propionibacteria at the rate of 0.2%. A paraffin-petroleum jelly plug was placed over the broth, and the inoculated media were incubated at 3.8, 10, 21, and 32 C for 4 months, 4 months, 4 days, and 2 days, respectively. The distances that the paraffin plugs were forced up the necks of the bottles by the gas produced were recorded.

Table 1. Strains of *Propionibacterium* spp. used in the investigation

Strain designation	Name of culture	Original strain designation	Source[a]
1	*P. freudenreichii*	F24	A
3	*P. pentosaceum*	E14	A
4	*P. thoenii*	TH25	A
5	*P. pentosaceum*	E214	A
15	*P. thoenii*	TH20	A
19	*P. shermanii*	KP1	C
24	*P. shermanii*	KP2	C
28	*P. pentosaceum*	167	B
30	*P. freudenreichii*	1291	E
31	*P. shermanii*	1294	E
33	*P. shermanii*	1298	E
35	*P. peterssonii*	1505	E
37	*P. shermanii*	6	F
39	*P. freudenreichii*	3	F
40	*P. freudenreichii*	5	F
42	*P. arabinosum*	10	F

46	*P. jensenii*	E.1.2.	G
47	*P. shermanii*	E.11.2	G
48	*P. shermanii*	E.11.3	G
50	*P. arabinosum*	E.7.1	G
51	*P. shermanii*	E.11.1	G
53	*P. peterssonii*	E.5.2	G
54	*P. jensenii*	E.1.1	G
55	*P. shermanii*	E.1.4	G
59	*P. shermanii*	ISU	B
74	*P. zeae*	PZ99	H
82	*P. shermanii*	Fin	D
83	*P. shermanii*	Sauer	B
105	*P. rubrum*	4871	B
109	*P. shermanii*	KP16	C
129	*P. arabinosum*	ISU9	B

[a]Source: A = Cornell University, Ithaca, N.Y.; B = Iowa State University, Ames, Iowa; C = Kraft Foods Co., Stockton, Ill.; D = Isolated from imported Emmentaler cheese; E = Dr. K. W. Sahli, Station Federale D'Industrie Laitiere, Liebefeld-Bern, Switzerland; F = Dr. V. B. D. Skerman, University of Queensland, Brisbane, Australia; G = Dr. C. B. van Niel, Hopkins Marine Station, Pacific Grove, Calif.; H = Communicable Disease Laboratory, Atlanta, Georgia.

Growth of Propionibacteria at Low Temperatures

To determine growth of propionibacteria at low temperatures, tubes containing 10 ml each of sterile Sodium lactate broth were preincubated at 3.8, 6.8, 10, and 15 C to preadjust temperatures before inoculation. These tubes were removed from incubation and immediately inoculated with various strains of Propionibacterium spp. at a rate of 0.1%. They were sealed with sterile paraffin wax, and returned to their respective incubators. The tubes were examined for growth and turbidity at 4, 7, 11, 17, 21, 28, 37, 59, and 78 days and the results recorded.

Growth Rate of Propionibacteria

To determine the proper time for harvesting the cells of strains of Propionibacterium spp., growth curves were established using a glucose broth: glucose 10 g, Trypticase (Baltimore Biological Laboratory) 10 g, yeast extract (Difco) 10 g, dibasic potassium phosphate 0.25 g, and distilled water 1000 ml, incubated at 32 C. A 0.06% inoculum was transferred to the broth and, at selected time intervals, a 3-ml sample was aseptically withdrawn from each flask and the optical density of the culture was determined on a Bausch and Lomb Spectronic 88 spectrophotometer (Bausch and Lomb, Inc., Rochester, New York) at 600 nm against a blank. Cultures to be tested were selected on the basis of growth or lack of growth at low temperatures.

To determine the growth rate of Propionibacterium shermanii 59

and Propionibacterium arabinosum 129, growth curves were established using Sodium lactate broth at 21 C. The broth was inoculated at a rate of 0.1%, samples were aseptically withdrawn at 1-hr intervals, and the cell numbers determined by the pouch technique of Hettinga et al. (51).

Preparation of Cell-Free Extracts of Propionibacteria

After three successive daily transfers of a rapidly growing strain of a Propionibacterium spp. in glucose broth, a 0.6% inoculum was transferred to 10 liters of glucose broth and incubated for 24 hr at 32 C. In most cases, 24 hr were sufficient to obtain luxuriant growth of the culture. Cells were harvested in a continuous flow Sorvall RC2-B automatic refrigerated centrifuge (Ivan Sorvall, Inc., Newtown, Connecticut) at 29,000 g and at 4 C. The time required for harvesting cells depended upon volume of broth inoculated. Harvested cells were dispersed in 10 x volume of 0.1 M, pH 8.0 potassium phosphate buffer, then centrifuged at 37,000 g, and again resuspended in fresh potassium phosphate buffer. This process of washing cells was repeated three times. Final suspension of cells in potassium phosphate buffer was at a 3 x concentration to obtain a thick slurry of cells. The cell slurry was subjected to 18,000 psi pressure in a French pressure device (Aminco, Inc., Silver Springs, Maryland) to obtain initial disruption of cells. Two passages through this pressure device at 18,000 psi accomplished good disruption of cell material. To insure

a more complete disruption of cell material, the cell slurry was subjected to 5 min of maximum sonication in a 10 kc Raytheon sonic oscillator (Raytheon, Waltham, Massachusetts). Ballontini glass beads, 45-90 u (Sigma Chemicals, Inc., St. Louis, Missouri), were added to the slurry to aid the disruption by sonication. Separation of the cell-free extract (CFE) from cell debris was accomplished by centrifugation at 37,000 g at 4 C. The cell-free extract was decanted from the cell debris and separated into 1- to 2-ml portions and placed in screw-capped test tubes. The cell-free extract was stored at -20 C until used for analysis.

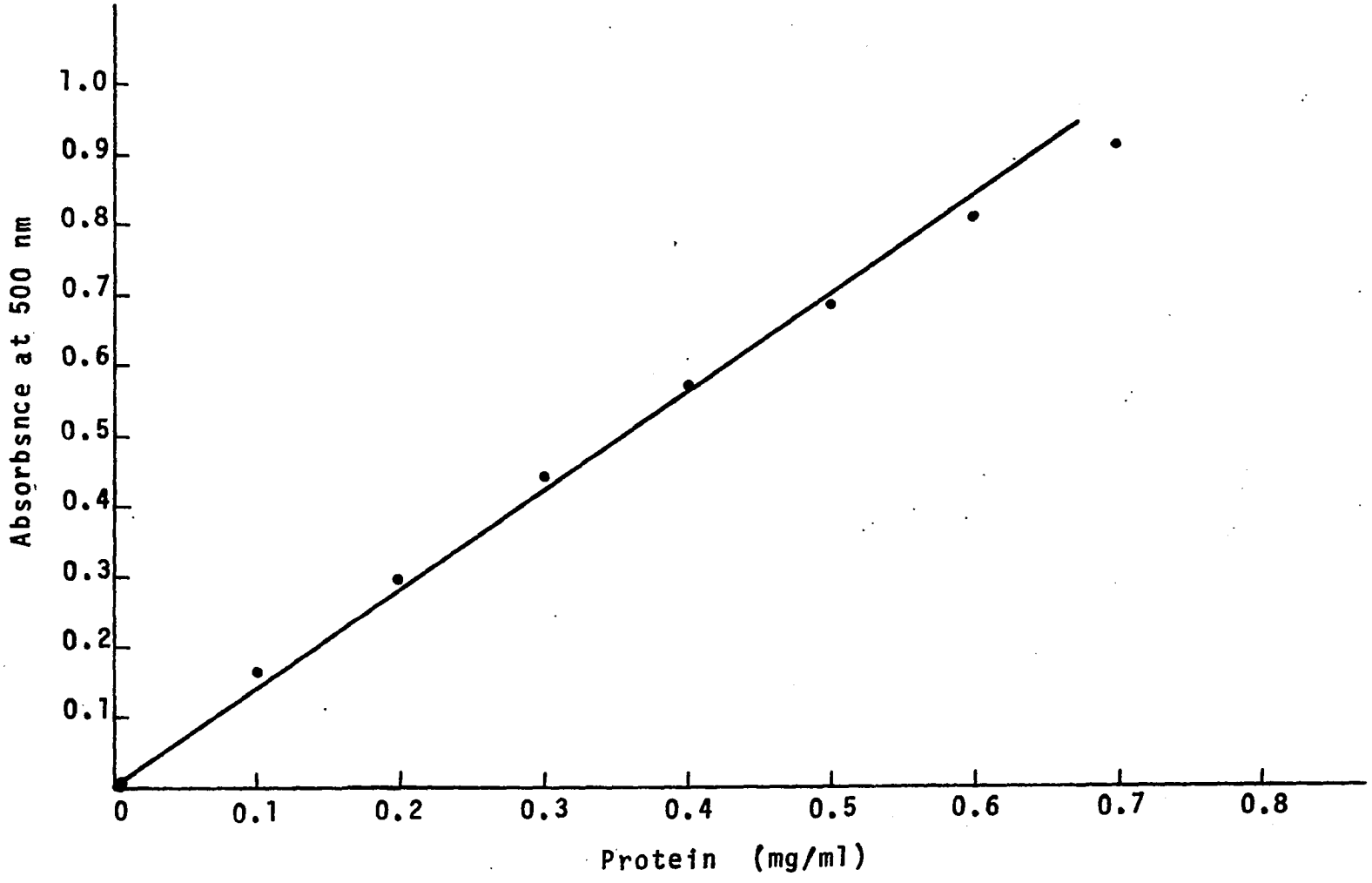
Determination of Total Protein

The method of Lowry et al. (77) was used to determine the protein content of the cell-free extracts. The standard curve was prepared using a known protein concentration of bovine serum albumin (Figure 6). Sodium citrate, instead of sodium or potassium tartrate, was used to dissolve the copper sulfate.

Preparation of Buffers

Potassium phosphate buffer was prepared by mixing 0.2 M stock solutions of monobasic and dibasic salts. Tris buffer was prepared by adding HCl to tris until the desired pH was obtained. All buffers were 0.1 M (41).

Figure 6. Relationship between absorbance and protein concentration


Absorbsnce at 500 nm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Protein (mg/ml)

Determination of Enzyme Activity

Table 2 lists the concentration and source of chemicals used in the following enzymatic studies.

Heat treatment of cell-free extracts

Cell-free extracts were heated in an oil bath using the following treatments: no heat (control); 5 min at 60 C; 10 min at 60 C; 5 min at 65 C; 5 min at 70 C; and, 5 min at 85 C. Activity of reduced nicotinamide adenine dinucleotide (NADH) oxidase and malate dehydrogenase was determined on each of the treated cell-free extracts (CFE) using a Bausch and Lomb Spectronic 20 spectrophotometer (Bausch and Lomb, Inc., Rochester, New York) at 340 nm.

Determination of NADH oxidase activity

The presence of NADH oxidase in cell-free extracts will affect the results of other enzyme systems producing NADH. To determine its activity, 3 ml of potassium phosphate buffer (0.1 M at pH 5.0, 6.0, 7.0, 7.5, 8.0, or 9.0), 0.1 ml CFE, and 0.1 ml of NADH were mixed together and the decrease in absorption at 340 nm was measured by a Bausch and Lomb Spectronic 20 spectrophotometer.

Isocitrate dehydrogenase activity

Three milliliters of double distilled water, 0.1 ml of CFE, 0.1 ml of 0.01 M $MgCl_2$, 0.1 ml of nicotinamide adenine dinucleotide phosphate, (NADP), and 0.1 ml of tris-citrate buffer 0.076 M pH 7.6, were mixed and the increase in absorption measured in a Bausch

Table 2. Concentration and source of chemicals used in the enzymic studies

Chemical	Abbreviation	Concentration	Source
Reduced nicotinamide adenine dinucleotide	NADH	5 mg/ml	Calbiochem, Inc., Fullerton, California
Nicotinamide adenine dinucleotide phosphate	NADP	5 mg/ml	Calbiochem
Glucose 6-phosphate disodium salt	glu-6P	0.025 M	Calbiochem
Oxaloacetic acid	OAA	0.25 M	Calbiochem
Nicotinamide adenine dinucleotide	NAD	5 mg/ml	Calbiochem
2p-iodophenyl-2p-nitrophenyl-5-phenyl-tetrazolium chloride	INT	1 mg/ml	Nutritional Biochem. Corp., Cleveland, Ohio
Phenazine-methosulfate	PMS	0.2 mg/ml	Calbiochem

Sodium pyruvate	Pyr	0.25 M	Calbiochem
Coenzyme A, lithium salt	CoA	3 mg/ml	Calbiochem
DL-thioctic acid (lipoic acid)		1 mg/ml	Calbiochem
Adenosine 5-triphosphate	ATP	1 mg/ml	Calbiochem
Thiamine pyrophosphate chloride	TPP	0.02 M	Calbiochem
Oxamic acid		0.5 M	Nutritional Biochem
Citrate condensing enzyme (porcine heart)	CCE	1000 units	Calbiochem
Lactic dehydrogenase (porcine heart)	LDH	2000 units	Calbiochem

and Lomb Spectronic 20 spectrophotometer at 340 nm.

Glucose 6-phosphate dehydrogenase activity

Three milliliters of tris-HCl or potassium phosphate buffer (0.1 M, pH 7.0), 0.1 ml of NADP, 0.1 ml of glucose 6-phosphate, 0.1 ml of $MgCl_2$ (0.01 M), and 0.1 ml of CFE were mixed together and the increase in absorption was measured at 340 nm with a Bausch and Lomb Spectronic 20 or Spectronic 88 spectrophotometer. Glucose 6-phosphate was added to initiate the reaction.

Malate dehydrogenase activity

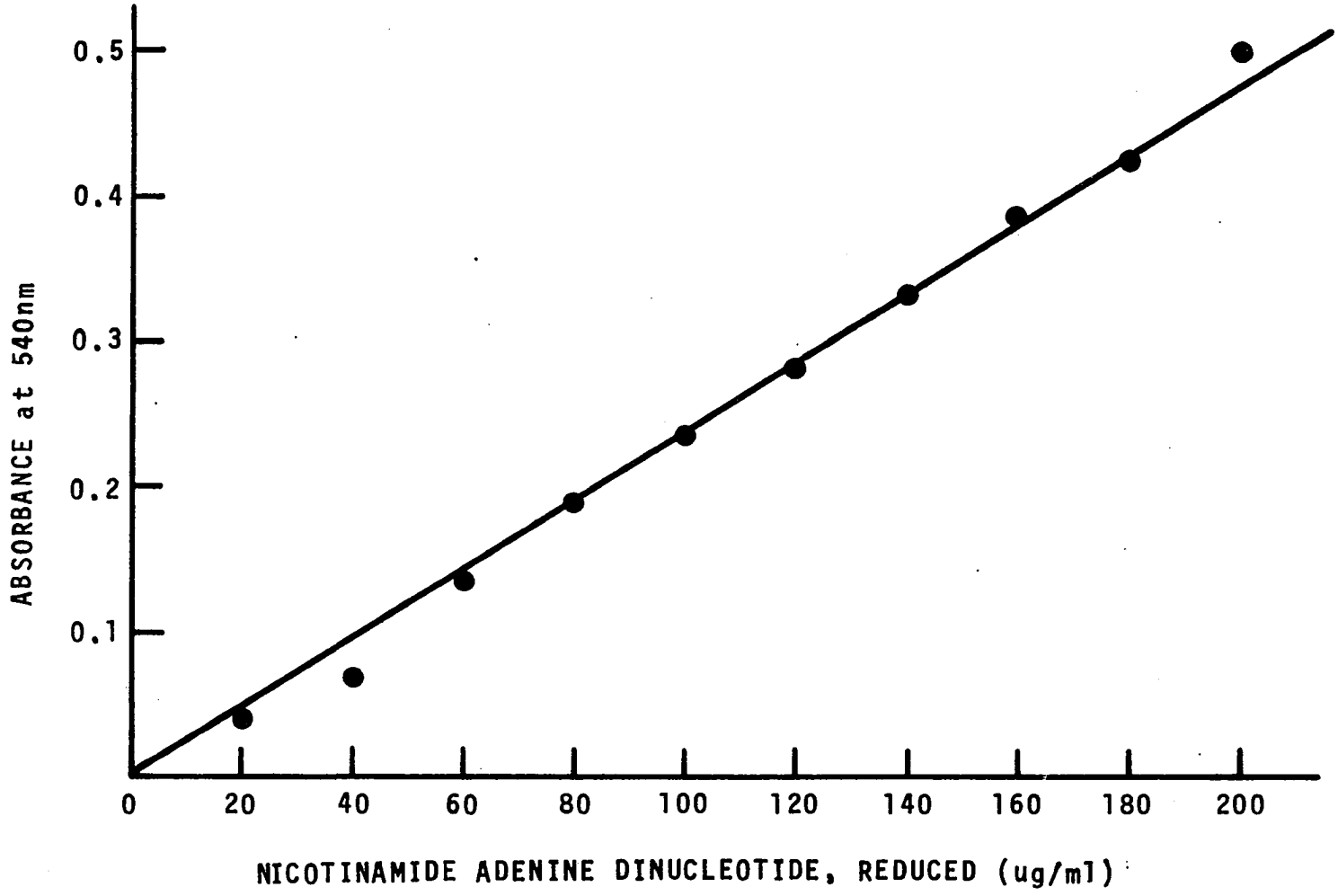
Three milliliters of potassium phosphate buffer (pH 7.5 or 8.0), 0.1 ml of CFE, 0.1 ml of oxaloacetic acid (pH 7.0), and 0.1 ml of NADH were mixed together and the decrease in absorption was measured by a Bausch and Lomb Spectronic 20, Spectronic 88, or a Beckman automatic scanning-recording spectrophotometer (Beckman Instruments, Inc., Fullerton, California) at 340 nm. The reaction was initiated by the addition of oxaloacetic acid.

Lactate dehydrogenase activity

The procedure consisted of adding 1.5 ml of buffered substrate (4.8 ml of 60% sodium lactate with 95.2 ml 0.1 M, pH 9.0 tris-HCl buffer), 0.5 ml of nicotinamide adenine dinucleotide (NAD, pH 7.0), 1.0 ml of 2p-iodophenyl-p-nitrophenyl-5-phenyltetrazolium chloride (INT), 0.5 ml of gelatin (0.1%), and incubating the mixture at the desired temperature. After the temperature of the sample had equilibrated, 0.5 ml of phenazine-methosulfate (PMS), and 0.5 ml

of CFE were added to the mixture and incubated for a reaction time of 15 min. The reaction was stopped by the addition of 0.5 ml of 0.35 N HCl which lowered the pH of the mixture to 4.5. Optimum pH for the reaction was 8.9 to 9.3. The mixture was transferred to optically matched cuvettes and the color read at 540 nm on a Bausch and Lomb Spectronic 88 spectrophotometer. The standard curve was prepared using either one of two procedures: 1) the concentrations of the mediator and tetrazolium were kept constant and the amount of reduced coenzyme was varied, plotting OD at 540 nm vs ug of NADH, or 2) the concentrations of the mediator and NADH were kept constant, and the concentration of tetrazolium added was varied, plotting OD at 540 nm vs µg INT. Both methods are justified as two reactants (INT and NADH) are required. Varying either reactant with the other in excess will limit the extent of the reaction (Figure 7). The blank was prepared by adding the acid to stop the reaction before the enzyme was added. A unit of lactate dehydrogenase activity is defined as that amount of enzyme that will yield 1 ug formazan under the conditions of assay. By using molar quantities it is possible to convert µg INT reduced to µ moles of pyruvate formed.

Figure 7. Relationship between absorbance and reduced nicotinamide adenine dinucleotide concentration


0.5
0.4
0.3
0.2
0.1
ABSORBANCE at 540nm
0
20
40
60
80
100
120
140
160
180
200
NICOTINAMIDE ADENINE DINUCLEOTIDE, REDUCED (ug/ml)

Pyruvate dehydrogenase activity

The methods used for determining pyruvate dehydrogenase activity in the CFE were as follows:

Spectrophotometric activity Three milliliters of potassium phosphate buffer (pH 7.5), 0.1 ml of CFE, 0.1 ml of sodium pyruvate, 0.1 ml of coenzyme A, 0.1 ml of thiamine pyrophosphate chloride (TPP), 0.1 ml of lipoic acid, 0.1 ml of NAD, 0.1 ml of $MgCl_2$, and 0.1 ml of adenosine 5-triphosphate (ATP) were mixed together and the increase in absorption was measured by Bausch and Lomb Spectronic 88 spectrophotometer at 340 nm. The reaction was initiated by addition of NAD, CFE, or pyruvate.

Colorimetric measurement of activity The procedure employed for the determination of lactate dehydrogenase activity was used with the following modification: pyruvate (0.5 M), coenzyme A (5mg/ml), and TPP (0.02 M) were substituted for sodium lactate as the substrate.

Manometric measurement of activity The dismutation assay described by Korkes (71) was used for the measurement of carbon dioxide liberation. Phosphotransacetylase was not added to the reaction mixture. Carbon dioxide liberation was measured by a Warburg apparatus (American Instrument Co., Silver Springs, Maryland) at 28 C.

Measurement of acetyl phosphate formation The dismutation assay described by Reed and Willms (101) was used for the measurement of acetyl phosphate by means of hydroxamic acid method.

Phosphotransacetylase was not added to the reaction mixture. The mixture was transferred to optically matched cuvettes and the color read at 540 nm on a Bausch and Lomb Spectronic 88 spectrophotometer.

RESULTS AND DISCUSSION

The Swiss cheese used in this investigation was manufactured with only one modification of the usual make procedure. This modification was the use of different strains of propionibacteria. In most cases, a comparison was obtained by manufacturing two lots of cheese under similar conditions at the same time using Propionibacterium shermanii strain 59, a known splitter, in one lot of cheese and Propionibacterium arabinosum strain 129, a nonsplitter in the other lot. After analysis of preliminary data was manufactured utilizing other strains of propionibacteria that may have possessed or lacked the ability to produce splits in Swiss cheese. The selection of these other cultures was based on the individual strain's ability or lack of ability to grow at low temperatures under laboratory cultural conditions. The cheese lot designations and the strains of Propionibacterium spp. used in this investigation are given in Table 15 of the Appendix.

Justification for consideration of growth at low temperatures as a criterion for propionibacterial strain selection was founded on the observation that splits in Swiss cheese are primarily a cold-room phenomenon. If a strain of Propionibacterium was able to metabolize lactate at low temperatures producing substantial amounts of carbon dioxide, and with the development of cheese inelasticity at low temperatures, a possible disposition for splitting may occur. Many factors could influence this tendency, but a thorough investigation of this hypothesis may result in the

detection of the causes of this defect.

Growth of Propionibacteria at Low Temperatures

An estimation of growth of strains of Propionibacterium spp. at various temperatures is shown in Table 3. Strains of 9 of the 11 recognized species of Propionibacterium were randomly selected and tested for their ability to grow at 3.8, 6.8, 10, and 15 C. The results showed that 8 of 32 strains were able to grow at 3.8 C, 17 of 32 strains at 6.8 C, 29 of 32 strains at 10 C, and that 32 of 32 strains grew at 15 C. Propionibacterium shermanii and P. freudenreichii, of the species tested, exhibited the greatest capacity to grow at low temperatures. Similar results were reported by Park et al. (94) where 3 of 49 strains were able to grow at 2.8 C, 28 of 49 strains at 7.2 C, and 47 of 49 strains grew well at 12.8 C. They also demonstrated that of all species tested, a greater proportion of the P. shermanii, P. freudenreichii, and and P. thoenii strains were able to grow at low temperatures.

Carbon Dioxide Production by Propionibacteria

Estimation of production of carbon dioxide by strains of Propionibacterium spp., selected for growth or lack of growth at low temperatures, in sodium lactate broth at various incubation temperatures is provided in Table 4. Carbon dioxide was produced when growth occurred and the quantity generally coincided with the rate of growth. Luxuriant growth and ample carbon dioxide production

Table 3. Estimation of growth of propionibacteria in sodium lactate broth at low temperatures

Propionibacterium spp.	Strain no.	Incubation time and temperature[a] 3.8 C 80 days	6.8 C 80 days	10 C 20 days	15 C 4 days
P. arabinosum	129	-[b]	-	-	+
P. arabinosum	42	-	-	-	+
P. arabinosum	50	-	-	+	+
P. freudenreichii	40	-	+	+	+
P. freudenreichii	39	+	+	+	+
P. freudenreichii	33	+	+	+	+
P. freudenreichii	30	-	-	+	+
P. freudenreichii	1	-	-	+	+
P. jensenii	46	-	-	+	+
P. jensenii	54	-	-	+	+
P. pentosaceum	5	-	-	+	+
P. pentosaceum	3	-	-	+	+
P. pentosaceum	28	-	-	-	+
P. peterssonii	35	-	+	+	+
P. peterssonii	53	-	-	+	+
P. rubrum	105	-	-	+	+

[a]Growth trials performed in duplicate.

[b]- = negative growth.
+ = definite growth.

Table 3. (Continued)

Propionibacterium spp.	Strain no.	Incubation time and temperature[a] 3.8 C 80 days	6.8 C 80 days	10 C 20 days	15 C 4 days
P. shermanii	83	+	+	+	+
P. shermanii	59	+	+	+	+
P. shermanii	19	-	+	+	+
P. shermanii	37	-[b]	+	+	+
P. shermanii	32	-	+	+	+
P. shermanii	47	+	+	+	+
P. shermanii	109	+	+	+	+
P. shermanii	51	+	+	+	+
P. shermanii	55	-	-	+	+
P. shermanii	31	-	+	+	+
P. shermanii	82	-	+	+	+
P. shermanii	24	-	+	+	+
P. shermanii	48	+	+	+	+
P. thoenii	15	-	-	+	+
P. thoenii	4	-	+	+	+
P. zeae	74	-	-	+	+

Table 4. Growth and carbon dioxide production by strains of Propionibacterium spp. at various temperatures of incubation

Strain no.[b]	Incubation time and temperature[a]							
	32 C, 4 days		21 C, 4 days		10 C, 90 days		3.8 C, 90 days	
	Growth	ml CO_2[c]	Growth	ml CO_2	Growth	ml CO_2	Growth	ml CO_2
83	+[d]	2.0	+	1.0	+	1.8	+	0.8
51	+	1.7	+	0.7	+	1.6	+	0.2
109	+	2.0	+	0.6	+	1.0	+	0.1
48	+	1.8	+	0.5	+	1.8	+	0.7
33	+	1.5	+	0.6	+	1.5	+	0.2
39	+	1.4	+	0.3	+	1.1	+	0.5
59	+	1.9	+	0.4	+	1.7	+	0.1
47	+	1.7	+	1.0	+	1.9	+	0.9
5	+	2.0	+	0.1	-	0	-	0
129	+	1.8	+	0.1	-	0	-	0
42	+	2.0	+	0.1	-	0	-	0

[a]Growth and carbon dioxide production determined in sodium lactate broth.

[b]The first eight strains were selected on the basis of growth at low temperatures. The last three strains were selected on the basis of lack of growth at low temperatures.

[c]Volume of broth displaced on a calibrated column. All figures are averages of duplicate determinations.

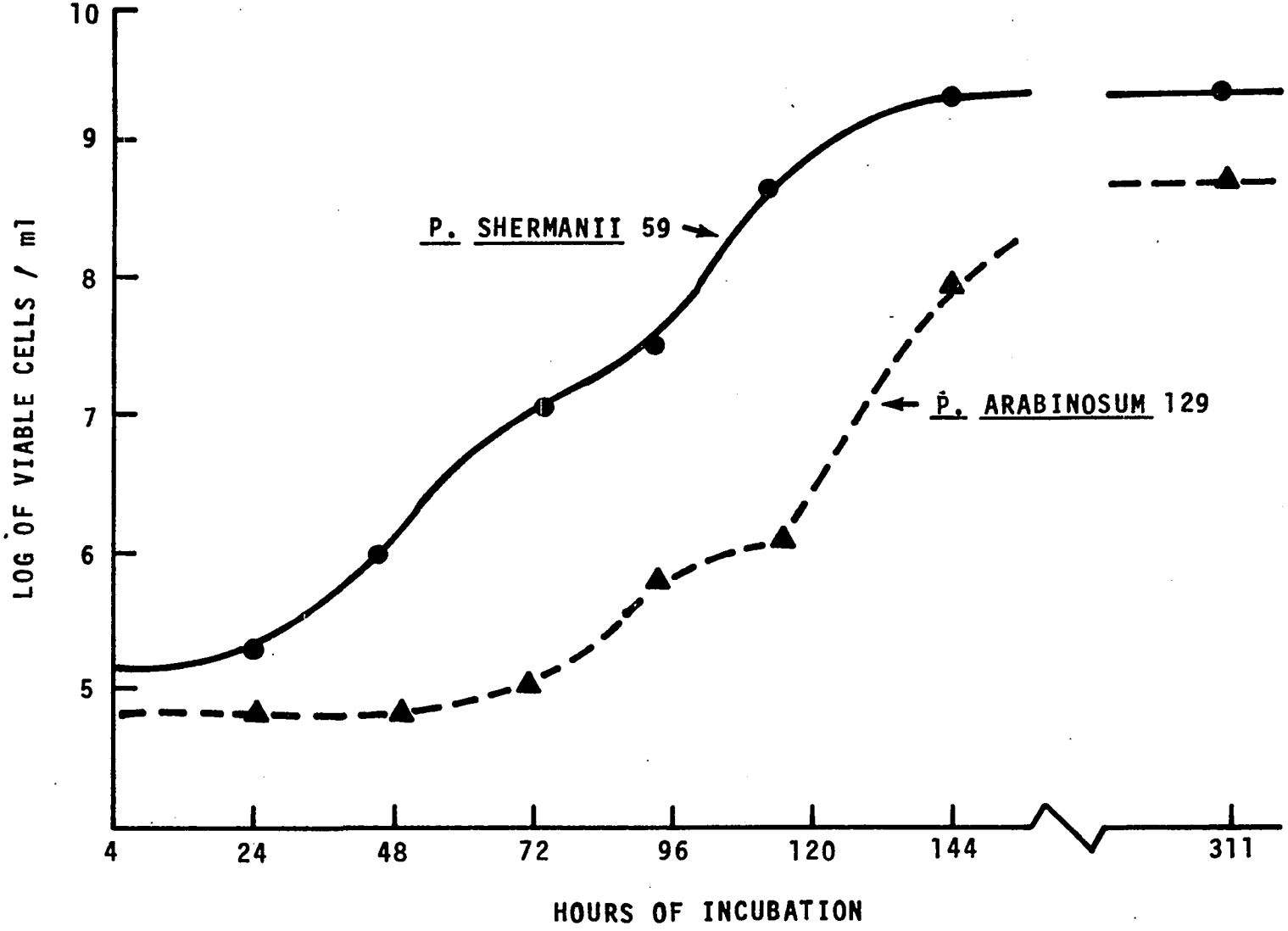
[d]+ = definite growth.
- = no growth.

was produced by all strains at 32 C. When the temperature was decreased to 21 C, the strains (P-5, P-42, and P-129), selected for lack of growth at low temperatures, exhibited equivalent growth but produced lower amounts of carbon dioxide. At 10 and 3.8 C, these same strains (P-5, P-42, and P-129) did not grow and subsequently produced no carbon dioxide. Propionibacterium shermanii strains 47 and 83 produced greater amounts of carbon dioxide at 3.8 C than all other strains selected for growth at low temperatures.

Growth Rate of Propionibacterium shermanii 59 and Propionibacterium arabionsum 129

Propionibacterium shermanii 59 and P. arabinosum 129 were the two strains of primary importance in this investigation. Their total number and rate of growth in pure culture could possibly provide insight as to their behavior in cheese. Figure 8 illustrates growth curves of these two strains. Propionibacterium shermanii 59 grew more rapidly and to higher total numbers than did the P. arabinosum 129 strain. The incubation temperature for establishing the growth curves was 21 C, which was approximately the same temperature used for hot-room curing of Swiss cheese. The rapid growth and higher numbers of the P-59 strain was indicative of the greater metabolic capability of this strain at this temperature. If P-59 exhibited this effect in pure culture, then similar results might occur in cheese.

Figure 8. Growth curves of Propionibacterium shermanii 59 and Propionibacterium arabinosum 129 in Sodium lactate broth at 21 C


10
9
8
7
6
5
LOG OF VIABLE CELLS / ml
P. SHERMANII 59
P. ARABINOSUM 129
4
24
48
72
96
120
144
311
HOURS OF INCUBATION

Occurrence of Splits in Experimental Swiss Cheese

Samples from each lot of cheese were visually observed at 3 and 6 months of age, and the results are shown in Table 5. The data provides information on the incidence of splits and checks in the experimental Swiss cheese. Of the 3-month old P-59 cheese, 4 of 9 lots (43%) were observed to contain checks but no splits. At 6 months of age, 5 of 9 lots (55%) of the P-59 cheeses showed splits and 8 of 9 lots (89%) contained checks. Splits or checks were not observed in 3-month old P-129 cheese at 3 months of age, but at 6 months, 1 of 7 lots (14%) were split and checked. In cheese made with strains that grew at low temperatures, 1 of 13 lots (8%) split and 6 of 13 lots (46%) were checked at 3 months of age. At 6 months, 10 of 13 lots (77%) of these cheeses had split and 12 of 13 lots (92%) were checked. Cheese manufactured with strains lacking the ability to grow at low temperatures did not split, but 1 of 10 lots (10%) was checked at 3 months of age. At 6 months, 2 of 10 lots (20%) were split and checked. These results support the hypothesis that propionibacteria that grow well at low temperatures have a greater disposition to produce cheese with splits and/or checks.

Growth of Propionibacteria in Cheese

Growth of lactic starters supersedes the initiation of growth of the propionibacteria in Swiss cheese. The growth of propionibacteria does not fully begin until the cheese enters the hot room. The propionibacteria then multiply rapidly, utilizing available

Table 5. The occurrence of splits and checks in Swiss cheese made with various strains of Propionibacterium spp.

Cheese lot[a]	Strain no.	Age of cheese			
		3 months		6 months	
		Splits	Checks	Splits	Checks
A	129	-[b]	-	-	-
B	59	-	+	+	+
C	59	-	-	-	+
D	129	-	-	+	+
E	59	-	-	+	+
F	129	-	-	-	-
G	59	-	+	+	+
H	129	-	-	-	-
J	35	-	-	-	-
K	1	-	+	+	+
L	129	-	-	-	-
M	83	-	-	+	+
O	59	-	-	+	+
P	83	-	+	+	+
Q	59	-	-	-	+
R	129	-	-	-	-
S	83	+	+	+	+
T	47	-	-	+	+
U	59	-	+	+	+
V	109	-	-	-	-
W	59	-	-	-	-
X	59	-	+	-	+
Y	129	-	-	-	-
Z	5	-	-	-	-

[a]The cheese lots in each group were manufactured at the same time and under similar conditions.

[b]- = negative.
+ = positive.

lactate, and produce the propionic acid, acetic acid, and carbon dioxide normally found in Swiss cheese. The hot room temperature of approximately 21 C, available lactate, and the physiological and chemical conditions within the cheese favor the growth of propionibacteria.

The comparison of the growth of two strains of propionibacteria from paired lots of Swiss cheese is illustrated in Figure 9. The P-59 strain increased more rapidly and attained higher numbers than did the P-129 strain. This observation was substantiated in all paired P-59 and P-129 lots of Swiss cheese as shown in Table 6. The higher numbers of P-59 cells coincide with the larger amounts of carbon dioxide produced and the greater consumption of lactate in the cheese. This observation substantiates results of Oehen et al. (89) where the increase in numbers of propionibacteria was paralled by increases in the volatile fatty acids and carbon dioxide production in Emmental cheese. Propionibacterial cell numbers increased rapidly during the hot room curing period and slowly decreased thereafter for the remaining cold room cure. The cheese was removed from the hot room when sufficient eye development had occurred. The decline in cell numbers generally coincides with the results of Sähli and Lehmann (111). They found that the number of propionic-acid bacteria declines from greater than 1 billion/g at 3 months to approximately 400 million/g at 6 months in high quality Emmental cheese.

All strains (P-39, P-47, P-59, P-83, P-109) tested for ability

Figure 9. Growth of Propionibacterium shermanii 59 and Propionibacterium arabinosum 129 during curing in experimental Swiss cheese

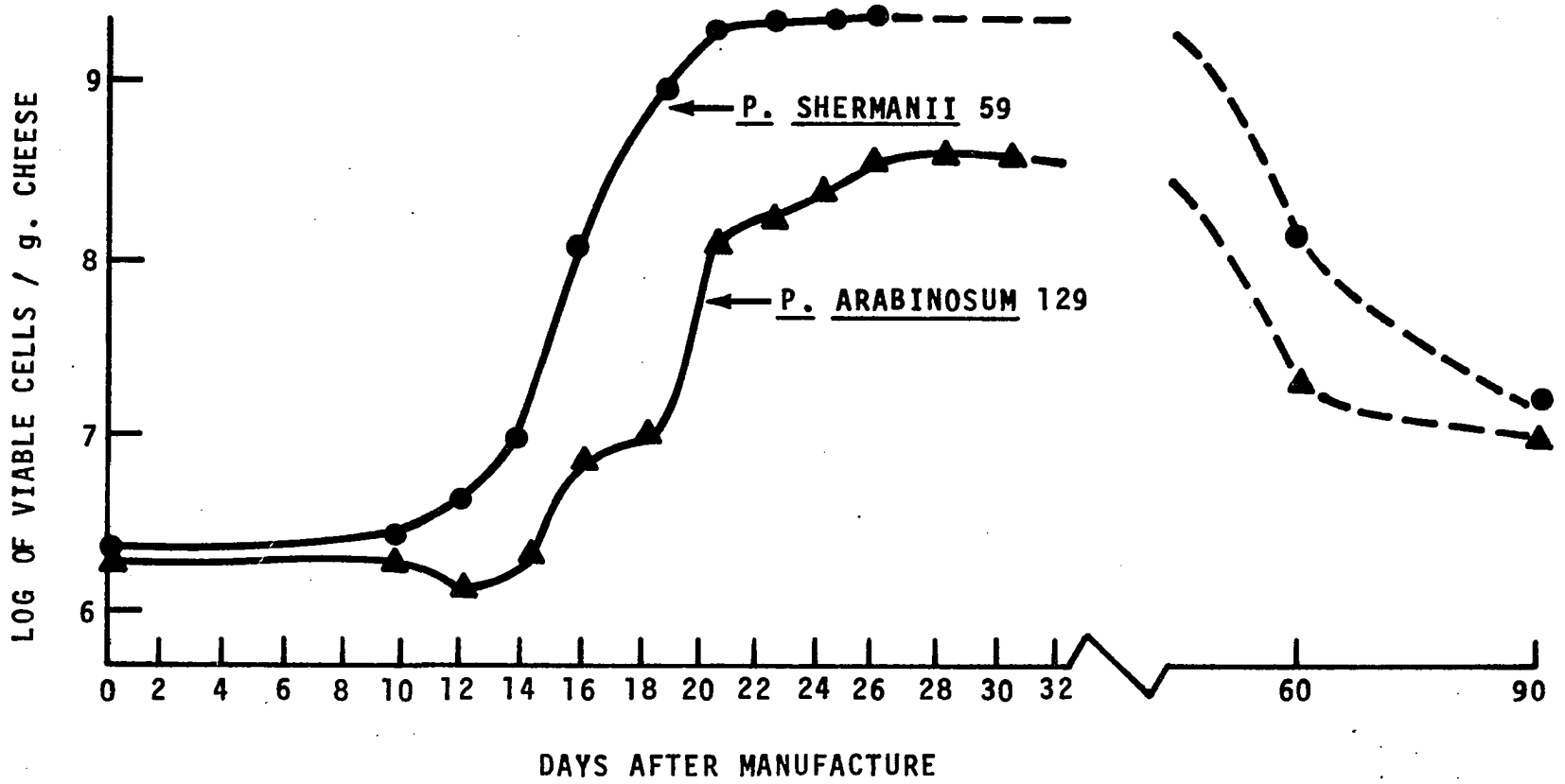

LOG OF VIABLE CELLS / g. CHEESE
9
8
7
6
P. SHERMANII 59
P. ARABINOSUM 129
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
60
90
DAYS AFTER MANUFACTURE

Table 6. Comparative determination of growth of strains of Propionibacterium spp. in Swiss cheese at various steps in the manufacturing and curing process

Cheese lot[a]	Strain no.	Stage of manufacturing and cure				
		In press	Into hot room	Out of hot room	3-months cure	6-months cure
		Viable cells/g of cheese (x 10^5)				
A	129	14[b]	20	5000	3520	540
B	59	40	4	20300	8800	560
C	59	12	4	18000	1360	400
D	129	156	5	2300	1650	830
E	59	41	66	15100	1410	210
F	129	20	38	6190	270	110
G	59	40	15	17300	1900	730
H	129	20	27	4200	760	400

J	35	63	24	24700	3800	920
K	1	35	80	4400	5000	850
L	129	69	92	2340	1260	270
M	83	41	89	10900	8700	650
T	47	50	50	56000	31300	16200
U	59	78	215	20800	1810	450
V	109	98	108	10300	18500	8500
X	59	110	375	18600	2500	630
Y	129	278	211	6500	1800	360
Z	5	511	460	2440	73	28

[a]The cheese in each group of lots were manufactured at the same time and under similar conditions.

[b]The figures represent averages of duplicate determinations.

to grow at low temperatures numerically exceeded those strains (P-1, P-5, P-129) that did not exhibit this ability. Several strains multiplied to exceptionally high numbers (2-3 billion/g) and persisted at these high levels (> 1 billion/g) toward the later stages of curing.

Disappearance of Lactic Acid in Cheese

Lactic acid is the primary carbon source for fermentation in the metabolism of propionibacteria in the curing of Swiss cheese. It was logical to analyze for lactic acid disappearance from the experimental cheese for determination of substrate availability. The disappearance of lactic acid for two strains of propionibacteria can be seen in Figure 10. It was observed that the disappearance of lactic acid closely correlates with the rate of carbon dioxide production and the increase in cell numbers of each strain. The P-59 strain rapidly fermented lactic acid, whereas, the P-129 strain did so at a much slower rate. Also, since larger increases in cell numbers and carbon dioxide production occurred with the P-59 strain, greater amounts of lactic acid were, thereby, fermented. At the end of 3 months of curing, the P-59 strain had utilized almost all available lactic acid. Since continued lactic acid disappearance had occurred during the cold-room cure of the cheese, it was indicative that bacterial metabolism may have been perpetuated during this period.

Table 7 shows the per cent lactic acid in cheese lots made

Figure 10. Disappearance of lactic acid during curing in experimental Swiss cheese manufactured with Propionibacterium shermanii 59 and cheese manufactured with Propionibacterium arabinosum 129

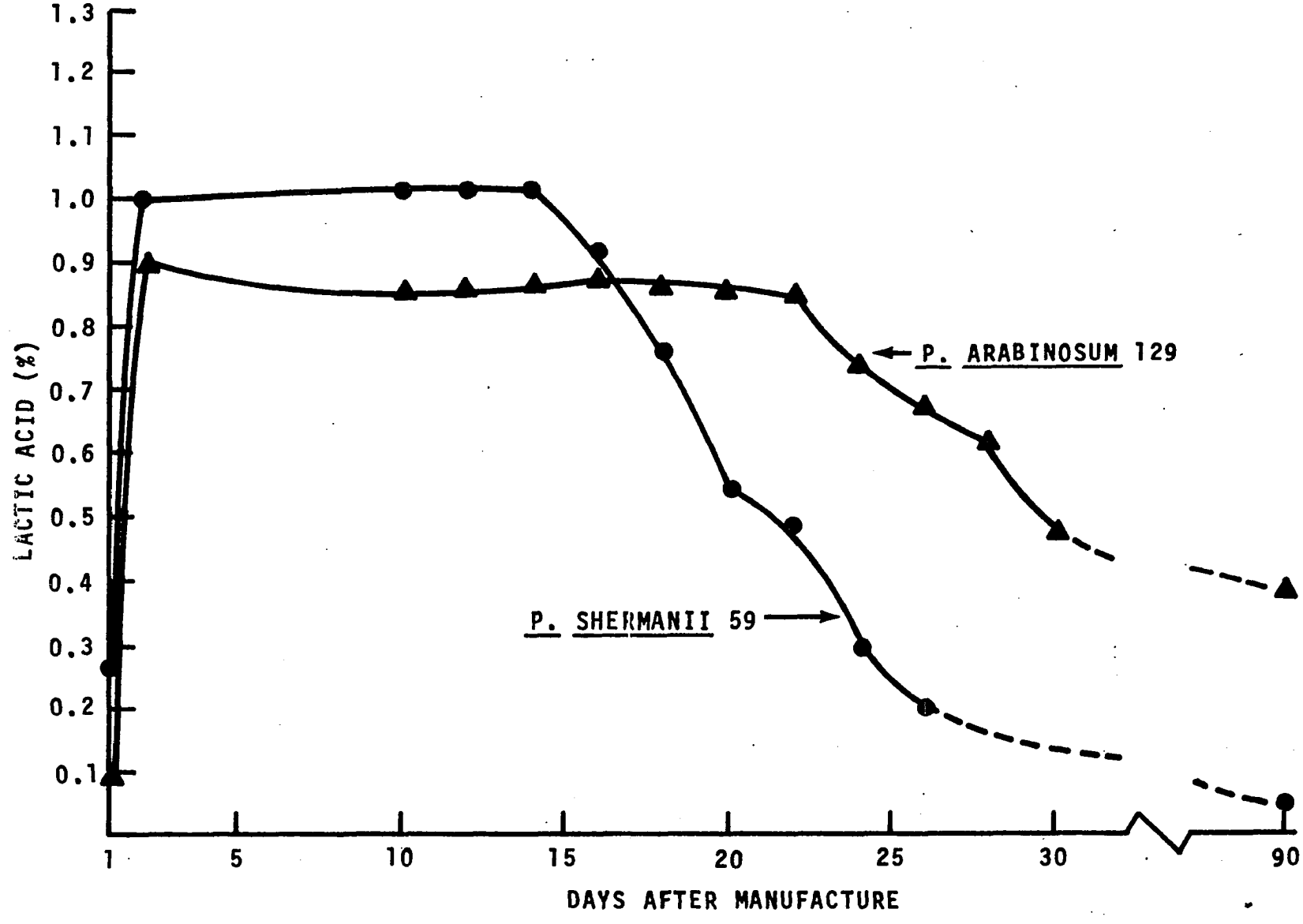

1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
LACTIC ACID (%)
P. ARABINOSUM 129
P. SHERMANII 59
1
5
10
15
20
25
30
90
DAYS AFTER MANUFACTURE

Table 7. Comparative determination of lactic acid in Swiss cheese at various steps in the manufacturing and curing process

Cheese lot[a]	Strain no.	Stage of manufacture and cure			
		During pressing	Into hot room	Out of hot room	3 months of cure
		Lactic acid (%)			
A	129	0.45	0.95	0.67	0.48
B	59	0.44	0.74	0.23	0.20
C	59	0.37	0.88	0.12	0.11
D	129	0.35	0.84	0.48	0.40
E	59	0.27	1.01	0.20	0.05
F	129	0.19	0.85	0.47	0.38
G	59	0.20	0.92	0.27	0.05
H	129	0.25	0.91	0.46	0.53
J	35	0.48	0.90	0.39	0.25
K	1	0.44	0.94	0.41	0.26
L	129	0.38	0.81	0.56	0.45
M	83	0.42	0.93	0.47	0.24
N	129	0.45	1.03	0.41	0.36
O	59	0.51	1.08	0.23	0.10
P	83	0.48	0.98	0.28	0.17
Q	59	0.45	1.05	0.17	0.08
R	129	0.47	0.78	0.53	0.47

[a]The cheese in each group of lots were manufactured at the same time and under similar conditions.

with various strains of propionibacteria and at different intervals of sampling. The data in Table 7 substantiate the results in Figure 10 when several trials using the same strains of propionibacteria were performed. It may be noted that the cheese

manufactured using P-83 behaved in a manner similar to the P-59 strain, especially since P-83 has shown its ability to produce splits and grow at low temperatures (Tables 3 and 5). The rate and amount of lactic acid utilized by the P-59 strain during the cold room cure was much greater than that fermented by the P-129 strain. This is a possible indication that greater metabolic activity occurred with the P-59 strain.

Eight lots of cheese were analyzed for lactic acid at the end of 6 months of curing, and no detectable amounts of lactic acid could be determined in cheese made with the P-59 strain, whereas, significant amounts (0.2%) of the acid were present in cheese made with the P-129 strain.

Proteolysis in Cheese

The extent of proteolysis was determined in eight lots of Swiss cheese. These lots consisted of four pairs of cheeses. Each pair was manufactured at the same time, under similar conditions, with but one variable in the manufacturing procedure. This variable was the strain of propionibacteria used in the manufacture of the cheese. The amount of dye bound/g of cheese decreased indicating a breakdown of the protein as the cheese aged. No significant differences in the degree of proteolysis could be observed between each pair of cheese lots. The per cent change, as proteolysis progressed, was calculated and variation appeared insignificant (Table 8). Chi square statistics were computed by comparing the

Table 8. Comparison of proteolysis in Swiss cheese manufactured with P. shermanii 59 and P. arabinosum 129 at various steps in the curing process

Cheese lot[a]	Strain no.	Stage of cure: Into hot room PI[b]	% [c]	Out of hot room PI	%	3-months cure PI	%	6-months cure PI
A	129	10.686[d]	9.61	9.659	7.10	8.973	4.79	8.543
B	59	11.073	10.25	9.937	8.51	9.091	3.50	8.772
C	59	11.218	9.61	10.139	14.17	8.702	2.68	8.468
D	129	11.199	10.89	9.979	14.40	8.542	2.08	8.364
E	59	11.000	7.68	10.155	20.28	8.095	2.32	7.907
F	129	10.676	14.49	9.129	11.60	8.070	2.52	7.886
G	59	11.132	8.01	10.240	13.74	8.833	2.51	8.606
H	129	10.397	10.65	9.289	13.53	8.032	2.45	7.835

[a]The cheese in each pair of lots were manufactured at the same time and under similar conditions.

[b]PI = Proteolysis index or the absorbancy units of orange G bound/g cheese.

[c]Per cent change between proteolysis indices.

[d]The PI figures represent averages of duplicate determinations.

four different periods of curing of P-59 cheese to P-129 cheese. The probability that the proteolysis indices were not significantly different was greater than 0.995. No correlation between the degree of proteolysis, the strain of propionibacteria used, and the incidence of splits existed. The results support the findings of Hammond and associates (43, 93) that proteolysis of Swiss cheese could not be correlated with splitting. The type of propionibacteria used in the manufacture of Swiss cheese has not been found to effect proteolysis to any extent (64).

The moisture and milk fat content of experimental Swiss cheese lots is given in Table 15 of the Appendix. The mean moisture values were statistically computed and found to be 41.92% for split cheeses and 42.02% for nonsplit cheeses. No significant differences in the moisture content were found between each pair of cheese lots. No correlation could be made between the amount of moisture and the incidence of splits and degree of proteolysis in the experimental Swiss cheese. It should be remembered that these moisture determinations were made on cheese before brining. Also, Swiss cheese made by the method used at Iowa State University is usually higher in moisture than cheese made by conventional commercial methods.

Persistence of Sugars in Cheese

Since starter cultures and many adventitious organisms are able to utilize lactose, glucose, and galactose in their metabolism, it was of interest to determine the amount of residual sugars in

cheese after manufacture. These sugars provide a fermentable carbohydrate source and could possibly play a role in production of unwanted carbon dioxide during the cold-room curing process.

Hettinga et al. (47) were able to demonstrate that detectable amounts of these sugars were present in 1-yr old Cheddar cheese. The results of analysis of 10 lots of Swiss cheese, used in this investigation, indicated that low levels of glucose and lactose and relatively high amounts of galactose were present when the cheese went into the press. Glucose (1.6 mg/g cheese) was approximately 1/10th the amount of galactose (14.8 mg/g cheese) recovered. Upon removal of the cheese from the press, and subsequent analysis of the cheese for presence of sugars, no glucose and only low amounts of galactose (0.2 mg/g cheese) were recovered. This indicated that the remaining sugars were metabolized, in the press, by the microorganisms present. This observation was indirectly confirmed by the subsequent increase in the amount of lactic acid and by the decrease in pH of the cheese during this pressing period.

The high amounts of galactose found confirms the report of Ritter et al. (106) that this sugar is metabolized after the fermentation of glucose and lactose. Also, it was noted that galactose can persist for 24 and for as long as 72 hr after manufacture (11). Because the method of manufacture of Iowa-type Swiss cheese involves extensive dilution of the whey with water, the low levels found could possibly account for the rapid disappearance of sugars present.

Estimation of Carbon Dioxide Production in Cheese

Production of carbon dioxide by propionibacteria is responsible for the development of eyes in normal Swiss cheese. An over-production, as well as a low production of this gas during the curing period, may produce a variety of defects in the cheese. Given the proper conditions, however, any microorganism that can produce sufficient gas could form eyes in Swiss curd. Once the process of eye formation is complete, the production of gas does not always terminate. The ability of certain propionibacteria to grow at low temperatures has been demonstrated (94). Since certain strains are able to grow at low temperatures, it is logical that their metabolism provides for additional carbon dioxide production at low levels. Splits in Swiss cheese are a cold-room phenomenon that may develop because of some chemical or physiological change that occurred within the cheese. One physiological change that occurs is that cheese loses much of the elasticity it had at hot-room temperatures, when it enters the cold room for final curing. Another change which could influence splitting is the additional (continuing) production of carbon dioxide creating an unnatural stress on the inelastic curd.

It is relatively simple to accurately measure carbon dioxide production by pure cultures growing in broth, but the artificial media devised so far do not approximate the relative power of cheese to maintain a more or less constant hydrogen ion concentration with the consequent extension of growth and control of enzymic

action, and to provide the proper degree of anaerobiosis. To study the actual production of carbon dioxide in cheese, it was necessary to develop a system which would collect all carbon dioxide diffusing from the cheese. This would provide an estimation of the relative ability of a selected propionibacterial strain to produce carbon dioxide. The system developed by the Food Technology Department, Iowa State University, is represented in Figure 3 of the Methods section. This system was used to estimate the relative ability of several strains of Propionibacterium, selected on the basis of growth or lack of growth at low temperatures, to produce carbon dioxide. Figure 11 illustrates the estimation of carbon dioxide production by P-59 and P-129 in two lots of Swiss cheese. Figure 12 shows the average carbon dioxide production of eight lots of P-59 cheese and eight lots of P-129 Swiss cheese. The P-59 strain produced larger amounts of carbon dioxide, developed eyes more rapidly, and maintained a higher level of carbon dioxide production throughout the cold room cure than did the P-129 strain.

Figure 13 illustrated the results of production of carbon dioxide by strains P-83 and P-47. These strains were selected on their ability to grow at low temperatures and on their capacity to produce large amounts of carbon dioxide in pure culture. Both strains demonstrated the ability to generate two or more times the amount of carbon dioxide than the average P-59 strain, and the cheese made with both strains produced splits (cheese lots S and T in Table 5). This ability to produce greater amounts of carbon

Figure 11. Carbon dioxide production during curing by 50 kg of experimental Swiss cheese manufactured with Propionibacterium shermanii 59 and by 50 kg of cheese manufactured with Propionibacterium arabinosum 129

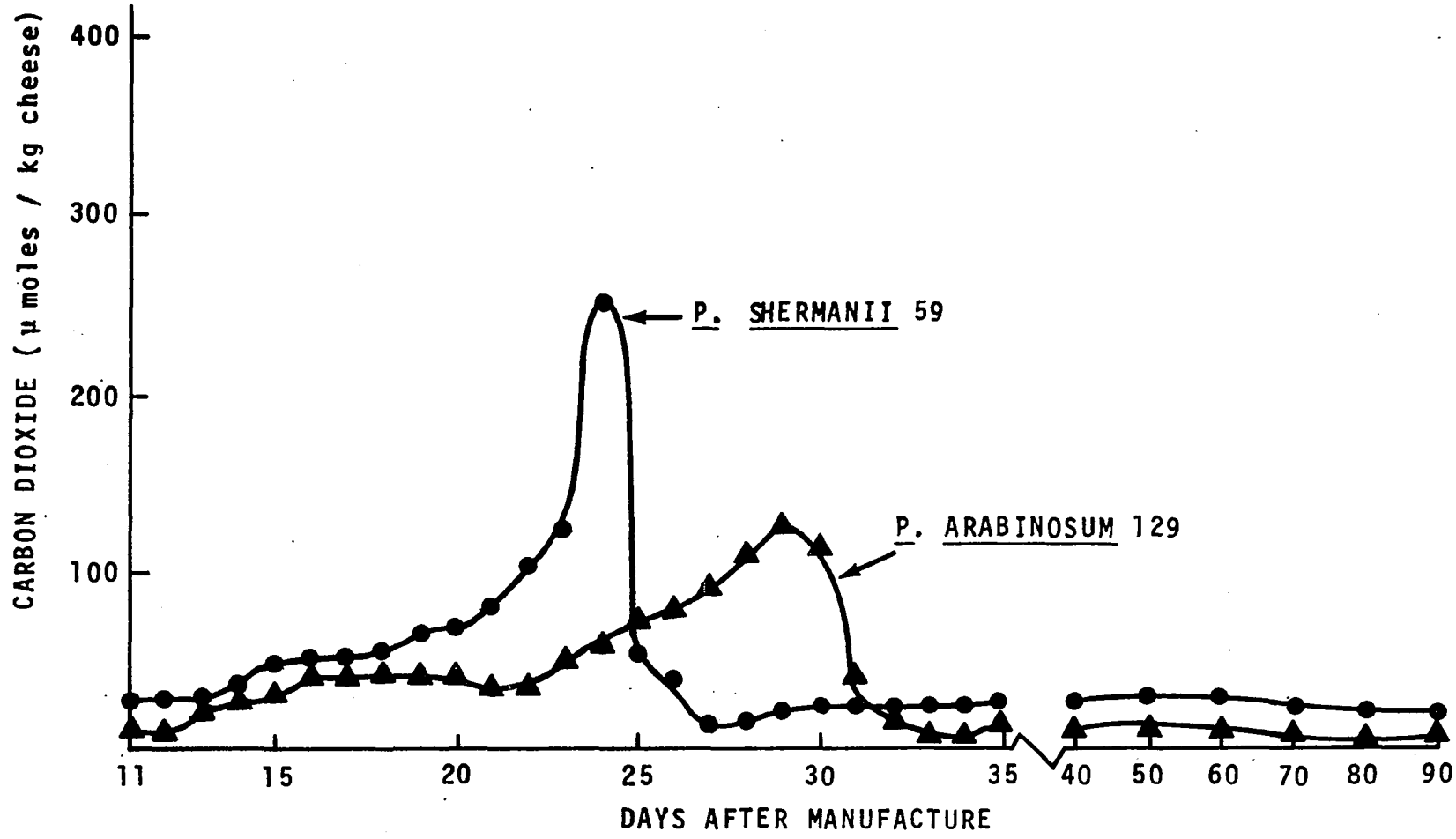

CARBON DIOXIDE (µ moles / kg cheese)
400
300
200
100
P. SHERMANII 59
P. ARABINOSUM 129
11
15
20
25
30
35
40
50
60
70
80
90
DAYS AFTER MANUFACTURE

Figure 12. Average carbon dioxide production during curing by eight lots of experimental Swiss cheese manufactured with Propionibacterium shermanii 59 and eight lots of cheese manufactured with Propionibacterium arabinosum 129

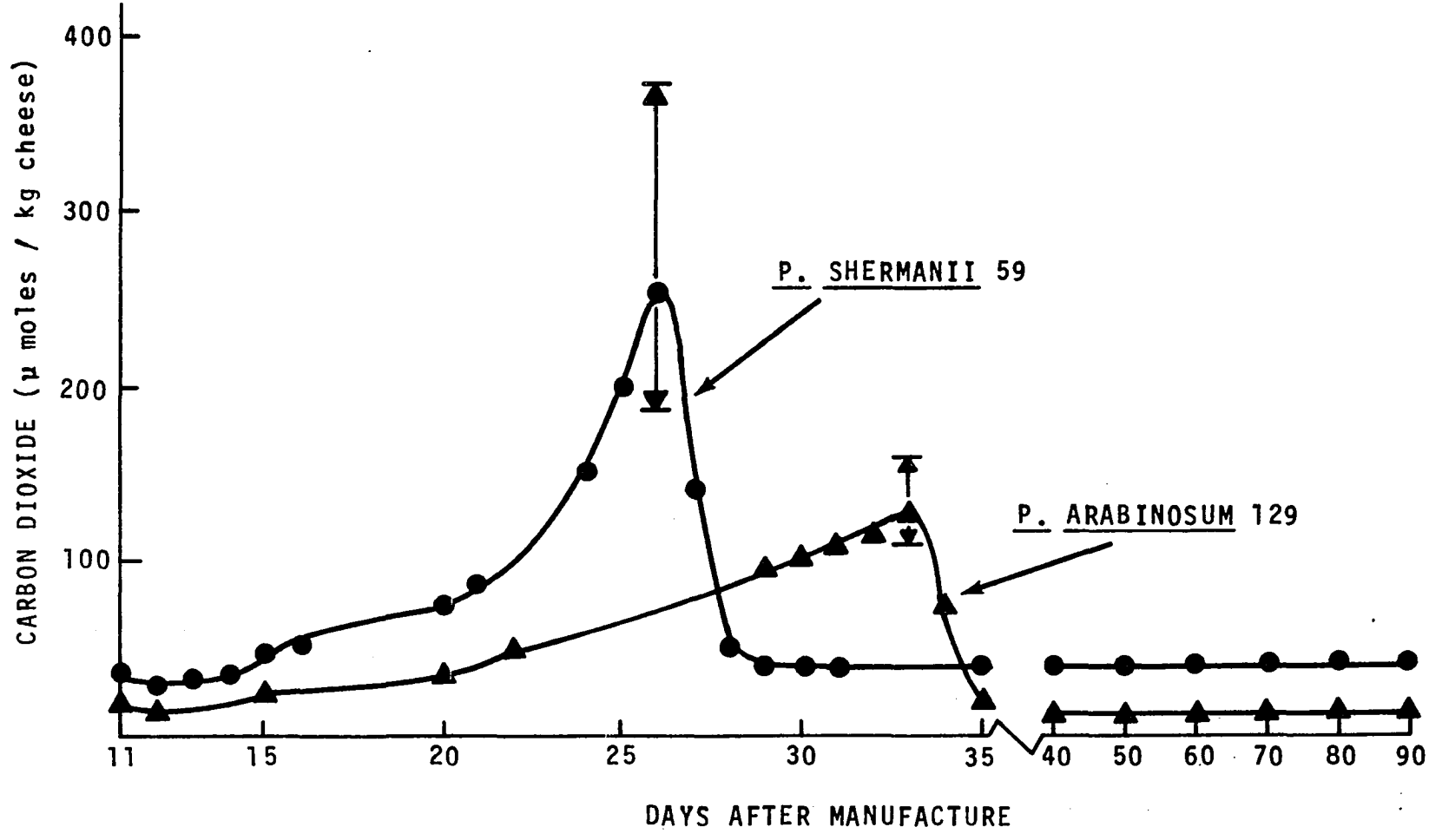
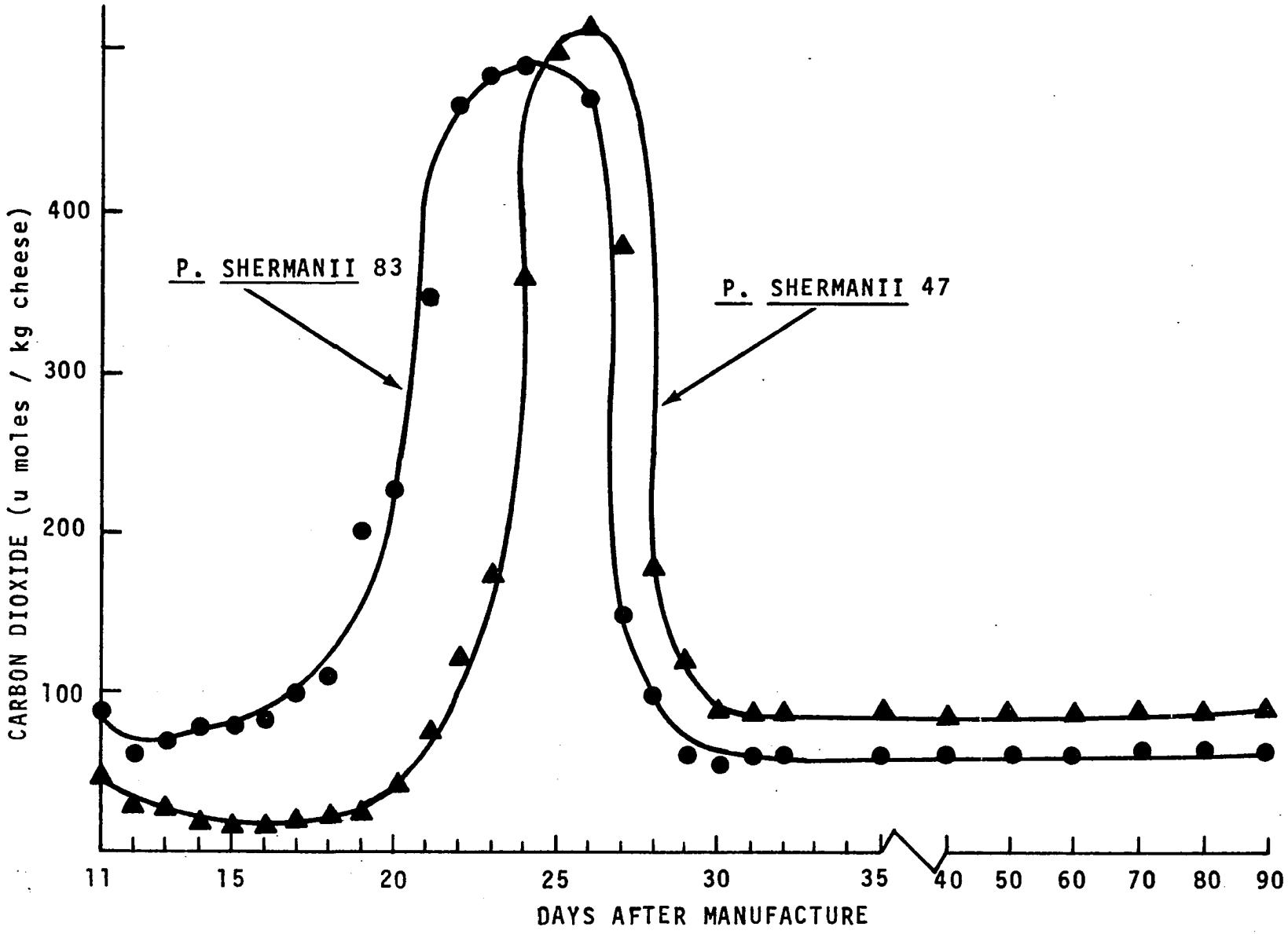

CARBON DIOXIDE (μ moles / kg cheese)
400
300
200
100
P. SHERMANII 59
P. ARABINOSUM 129
11
15
20
25
30
35
40
50
60
70
80
90
DAYS AFTER MANUFACTURE

Figure 13. Carbon dioxide production during curing by 50 kg of experimental Swiss cheese manufactured with Propionibacterium shermanii 47 and cheese manufactured with Propionibacterium shermanii 83


CARBON DIOXIDE (u moles / kg cheese)
400
300
200
100
P. SHERMANII 83
P. SHERMANII 47
11
15
20
25
30
35
40
50
60
70
80
90
DAYS AFTER MANUFACTURE

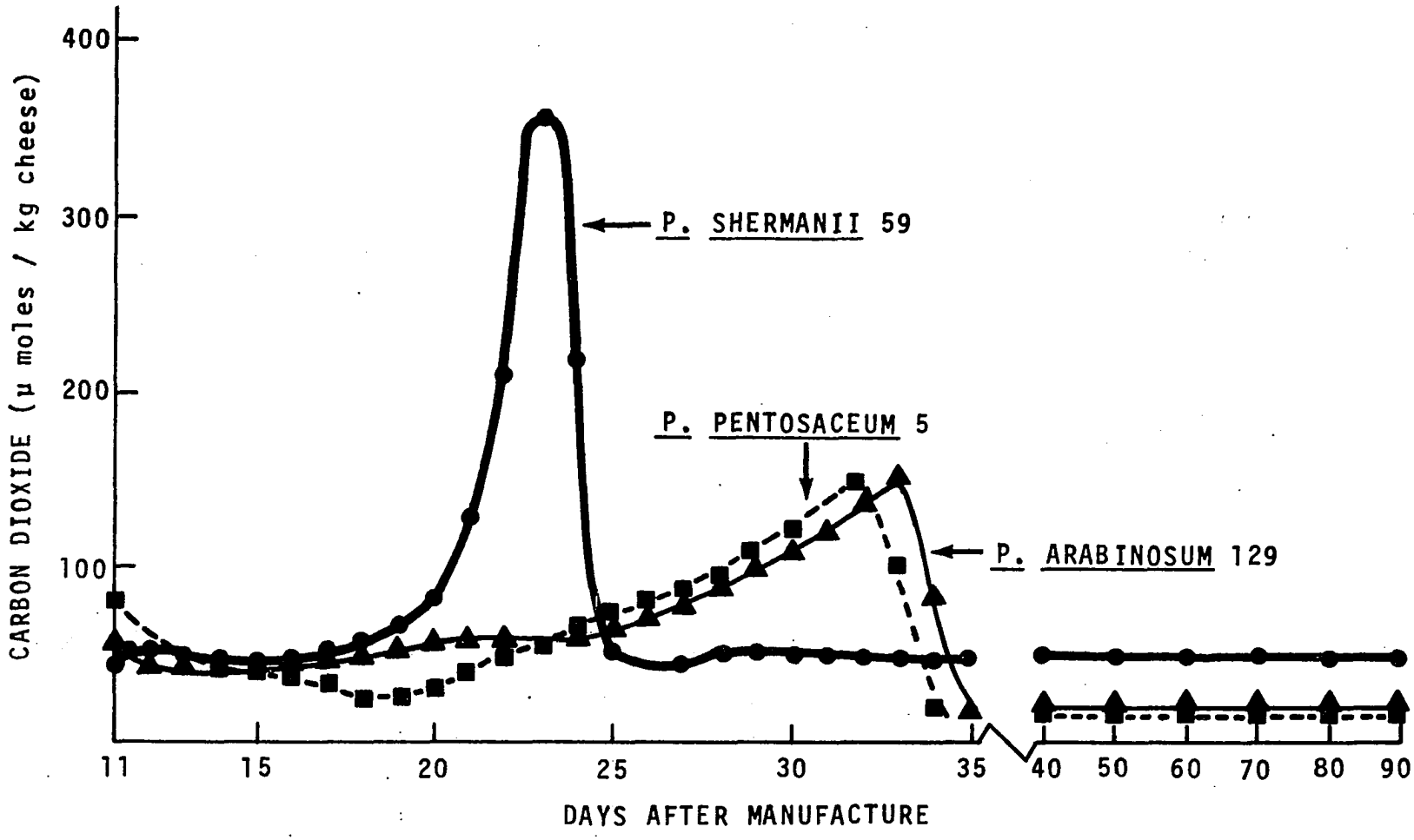
dioxide in cheese coincided with results obtained when P-47 and P-83 produced large amounts of carbon dioxide in pure culture (Table 2).

Figure 14 presents the carbon dioxide production by strains P-5, P-59, and P-129. Again, the P-59 and P-129 strains functioned in the usual manner. The P-5 strain, selected for lack of growth at low temperatures and a decreased ability to produce carbon dioxide, affected results very similar to those obtained by use of the P-129 strain. The cheese manufactured with the P-5 strain did not split (Table 5), which indicated and further substantiated the reliability of the criterion of culture selection on the basis of growth at low temperatures. The results of all lots of cheese tested for carbon dioxide production by various strains of Propionibacterium spp. are available for comparison in Table 16 in the Appendix.

Several investigators (55, 63, 107) have demonstrated that strains of lactobacilli and micrococci may stimulate carbon dioxide production by propionibacteria. This is a possible explanation why some cheeses split, whereas, other cheeses using the same cultures and manufactured under similar conditions may not.

The results of carbon dioxide production indicated that greater stress may have been produced by the P-59 and other strains that grow at low temperatures on the elasticity of the cheese body, resulting in a higher incidence of splits in the respective cheeses.

Figure 14. Carbon dioxide production during curing by 50 kg of experimental Swiss cheese manufactured with Propionibacterium shermanii 59, 50 kg of cheese manufactured with Propionibacterium arabinosum 129, and 50 kg of cheese manufactured with Propionibacterium pentosaceum 5


CARBON DIOXIDE (μ moles / kg cheese)
400
300
200
100
P. SHERMANII 59
P. PENTOSACEUM 5
P. ARABINOSUM 129
11
15
20
25
30
35
40
50
60
70
80
90
DAYS AFTER MANUFACTURE

Effect of Cheese Wrapping Films

Three experiments were conducted to determine the effect of wrapping films on the incidence of splits in Swiss cheese. Two of these experiments were performed on seven lots of experimental Swiss cheese and one experiment on four lots of commercial Swiss cheese. The first experiment was conducted on a comparison of four wrapping films on two lots of experimental Swiss cheese and the results are shown in Table 9. No definite correlation to the effect of wrapping films to the incidence of splits in this first experiment could be drawn from the data obtained. The Cryovac wrapped cheese (control, film I of Table 17 in the Appendix) showed the same results as cheese wrapped in Cryovac and paraffin (film II). The Cryovac-paraffin wrapped cheeses provided a presumably impermeable gas barrier. It was hypothesized that the cheese wrapped to provide an impermeable gas barrier would produce a cheese more likely to split. This hypothesis was supported by the results obtained by Kiuru and Uotila (62). They have shown that Emmental cheese with rind or cheese wrapped in a film more permeable to carbon dioxide was less susceptible to swelling and development of splits than cheese with an impermeable rind or cheese wrapped in films of poor carbon dioxide permeability. A reasonable explanation to the observations in Table 9 is that the Cryovac film used as a control provides, in itself, a near impermeable gas barrier. This may help to explain why some checks were observed in the P-59 wrapped in the control (film I) as well as in the Cryovac-paraffin (film

Table 9. Comparison of four wrapping methods on two lots of 6-month old experimental Swiss cheese with each lot manufactured with a different strain of propionibacteria

Wrapping film[a]	Comments on eye quality of 6-month Swiss cheese	
	P-59 cheese	P-129 cheese
I	Overset, good distribution of eyes, checks, no splits	Good distribution of eyes, no checks and no splits
II[b]	Overset, good distribution of eyes, checks, no splits	Good distribution of eyes, no checks and no splits
II[c]	Overset, good distribution of eyes, checks, no splits	Good distribution of eyes, no checks and no splits
II[d]	Overset, good distribution of eyes, collapsed, no checks and no splits	Good distribution of eyes, no checks and no splits

[a]Refer to Table 17 in the Appendix for type and properties of wrapping films.

[b]Single Cryovac wrap with paraffin overlayer.

[c]Same as film II in Table 17 in the Appendix.

[d]Cryovac-paraffin-Cryovac-paraffin (four layers).

II) wrapped cheeses. No checks or splits were observed in the P-129 cheese wrapped in any of the films. Correction of this wrapping film miscalculation was provided in the second experiment (Table 10). In the second experiment each cheese lot was divided into two equal portions and one-half of the blocks was wrapped in Cryovac (film I), the other half was wrapped in the Nealam film (film III). The Nealam (film III) was a special wrapping film providing relatively high carbon dioxide permeability and a low oxygen transmission rate (see Table 17 in the Appendix). No splits or checks were observed in all cheese at 3 months of age. A sorbate treatment was given to all blocks wrapped in Cryovac (film I), whereas, the Nealam (film III) blocks were untreated. Mold spots developed on the cheese wrapped in both films, but a greater tendency for the development of mold was observed on the blocks wrapped in Cryovac (film I). This mold development in the Cryovac wrapped blocks was possibly due to imperfections in the wrapping procedure. All cheeses had a tendency for the eyes to collapse. A possible explanation for this tendency to collapse is the method used in stacking the cheese during cold storage. The eyes were allowed to develop in the hot room beyond routine size, for oversetting was desired to aid the induction of splitting. This overset condition along with stacking the cheese one upon another without mechanical support may have placed too much stress upon the cheese body, thus the eyes collapsed. Similar results were obtained when the cheese lots were cut and observed

Table 10. Comparison of two different wrapping films on five lots of 3-month old experimental Swiss cheese with each lot manufactured with one of five strains of propionibacteria

Strain no.	Wrapping film[a]	Comments on eye development	Mold[b]	Split
109	I	Surface eyes, collapsed, overset	+	-
109	III	No surface eyes, overset, collapsed	-	-
5	I	Collapsed, overset	+	-
5	III	Collapsed, overset	-	-
83	I	Heavyset[c], small eyes	+	-
83	III	Heavyset, small eyes	-	-
129	I	Good eye distribution, overset	+	-
129	III	Good eye distribution, overset	+	-
59	I	Overset, distended blocks	-	-
59	III	Overset, square blocks	-	-

[a]Refer to Table 17 in the Appendix for type and properties of wrapping films.

[b]+ = mold growth on cheese surface.
- = no mold growth on cheese surface.

[c]Heavyset = too many eyes, not as severe as overset.

at 6 months of age.

The production and film transmission rates of carbon dioxide were determined on one lot of cheese wrapped in Cryovac and Nealam films (films I and III). Comparative results of the P. shermanii 59 split lot cheeses are illustrated in Figure 15. Carbon dioxide, as produced in the cheese wrapped in the Nealam film, showed a constant diffusion rate increase during the hot room cure and maintained a higher sustaining level during the cold room curing as compared to the Cryovac wrapped cheeses. This data supports the manufacturer's contention that the Nealam film readily diffuses carbon dioxide.

The third experiment involved a comparison of four different wrapping films on commercial Swiss cheese. Four lots of Swiss cheese were split into four equal portions and each portion was wrapped with a different film. Each lot of cheese was manufactured under identical conditions except for the strain of Propionibacterium spp. employed. The wrapping films used were rated on a gas transmissibility gradient scale from impermeable to highly permeable (see Table 17 in the Appendix). The results of the third experiment are shown in Table 11. The data shows that a cheese wrapped in a highly permeable film (film VI) has a decreased tendency to check or split as compared to cheese wrapped in impermeable films (films IV and VII). There was some inconsistency in the results when they were compared to the strain of Propionibacterium spp. used in the cheese manufacture. It was hypothesized that of the four strains

Figure 15. Carbon dioxide production by a split-lot of experimental Swiss cheese manufactured with Propionibacterium shermanii 59, 50 kg of cheese wrapped in semipermeable Cryovac SW clear 60 film and 50 kg of cheese wrapped in semipermeable Nealam 60954 film

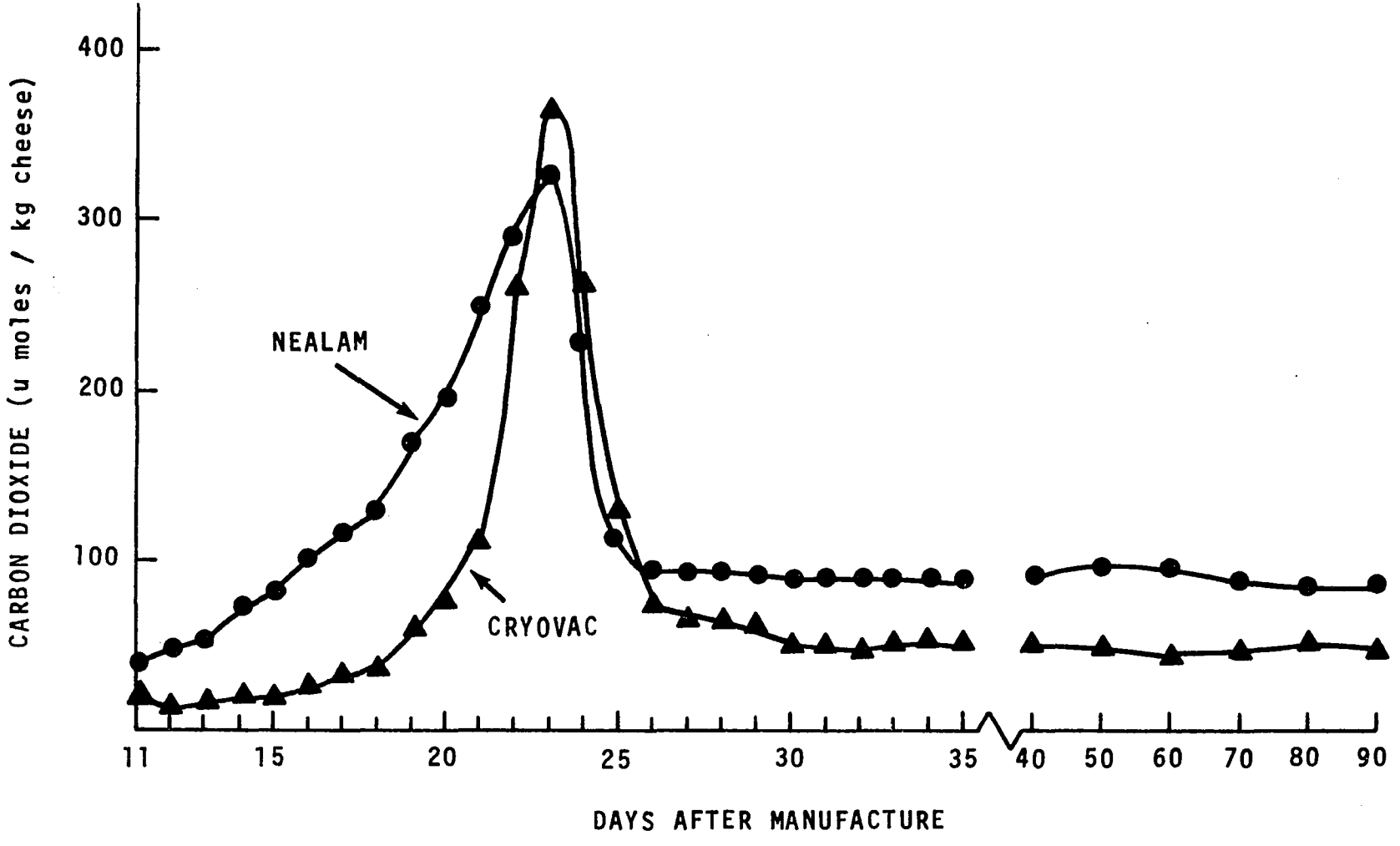

CARBON DIOXIDE (u moles / kg cheese)
400
300
200
100
NEALAM
CRYOVAC
11
15
20
25
30
35
40
50
60
70
80
90
DAYS AFTER MANUFACTURE

Table 11. Comparison of four different wrapping films on four lots of 3-month old commercial Swiss cheese with each lot manufactured with one of four strains of propionibacteria

Strain no.	Wrapping film[a]	Film permeability[a]	Comments on eye development[b]
59	IV	impermeable	Heavyset, no splits or checks
59	V	semi-permeable	Heavyset, no splits or checks
59	VI	permeable	Heavyset, no splits or checks, moldy
59	VII	impermeable	Heavyset, no splits or checks
83	IV	impermeable	Overset, high incidence of splits
83	V	semi-permeable	Overset, splits and checks
83	VI	permeable	Overset, checks, no splits, moldy
83	VII	impermeable	Overset, splits and checks
129	IV	impermeable	Overset, splits and checks
129	V	semi-permeable	Overset, splits and checks
129	VI	permeable	Overset, checks, no splits, moldy
129	VII	impermeable	Overset, high incidence of splits
5	IV	impermeable	Overset, checks, no splits
5	V	semi-permeable	Overset, no checks, no splits
5	VI	permeable	Overset, no checks, no splits, moldy
5	VII	impermeable	Overset, checks and splits

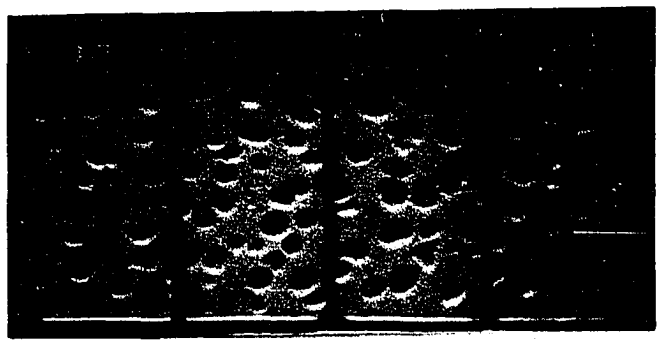
[a]Refer to Table 17 in the Appendix for type and properties of wrapping films.

[b]All four lots of cheese were graded "A" quality.

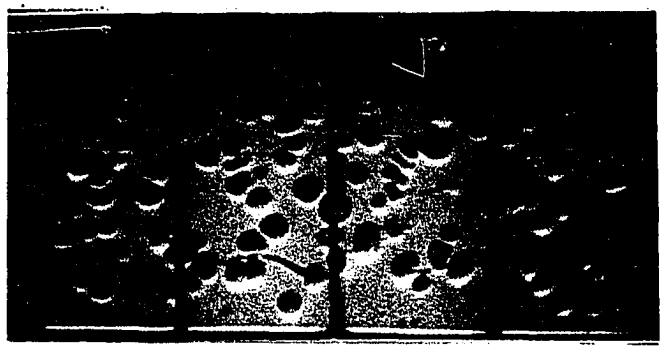
used, P-59 and P-83 would produce cheese with the highest incidence of checks and splits and the P-5 and P-129 cheese with no or low splitting tendencies. The results showed that the P-5 and P-83 cheese correlated with the hypothesis. The P-59 and P-129 cheese showed opposite results. An explanation to this inconsistency is that the two strain's identifying numbers were possibly reversed when handled or recorded. Since the handling of cultures and the manufacture of the cheese were performed in a commercial operation, no controls over these conditions could be employed. To check this possibility of strain number reversal, isolation of the Propionibacterium from a sample of the P-59 cheese and a preliminary identification of the microorganism was performed. This preliminary identification was performed to determine if the Propionibacterium from the P-59 cheese resembled P. shermanii by showing the ability to grow at low temperatures and the lack of ability to ferment sucrose. The results were negative thus indicating that the Propionibacterium did not resemble P. shermanii and that the cultures may have been reversed.

Figures 16 and 17 are photographs of commercial Swiss cheese manufactured with P-83 and P-5, respectively. As shown in Figure 16, the P-83 cheese produced splits and checks in all cheese wrapped in semi- and impermeable films (photos I, II, and IV), whereas, the cheese in the permeable film (photo III) was not split or checked. In Figure 17, the P-5 cheese showed a decreased tendency to split or check regardless of the film used. Both impermeable films used

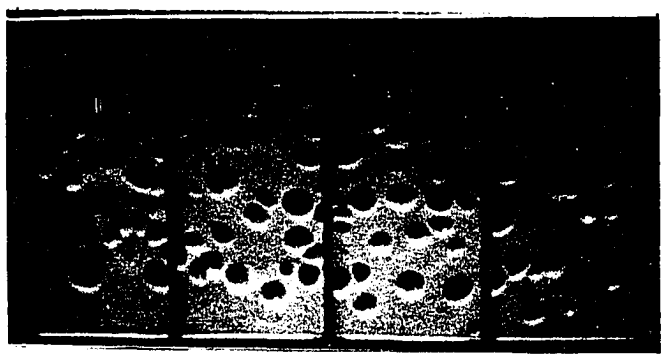
Figure 16. Photographs of the effect of four different wrapping films on four blocks of commercial Swiss cheese manufactured with Propionibacterium shermanii 83. Cheese I was wrapped with impermeable Saranex 29 duplex, cheese II with semipermeable Saranex 29 simplex and polyethylene, cheese III with permeable polyethylene duplex, and cheese IV with an impermeable polyester/Saran/polyethylene wrap



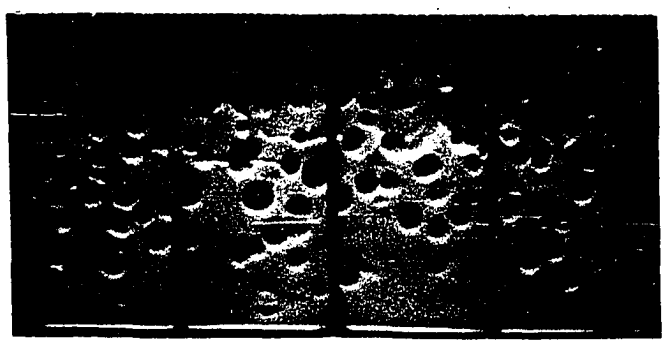
I



II

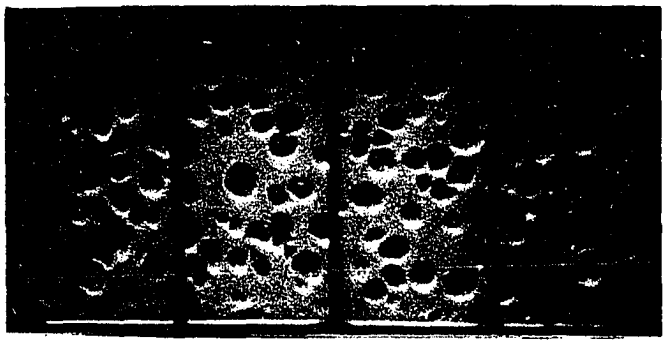


III

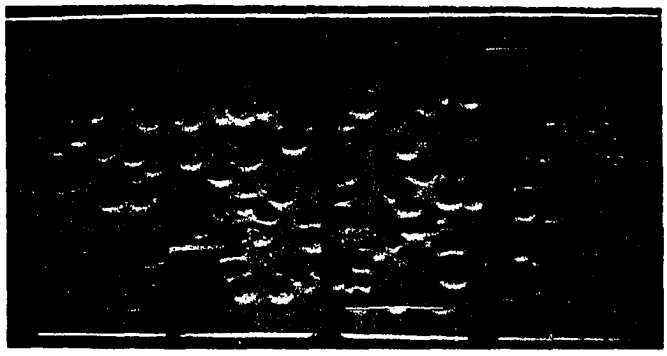


IV

Figure 17. Photographs of the effect of four different wrapping films on four blocks of commercial Swiss cheese manufactured with Propionibacterium pentosaceum 5. Cheese I was wrapped with impermeable Saranex 29 duplex, cheese II with semipermeable Saranex 29 simplex and polyethylene, cheese III with permeable polyethylene duplex, and cheese IV with an impermeable polyester/Saran/polyethylene wrap

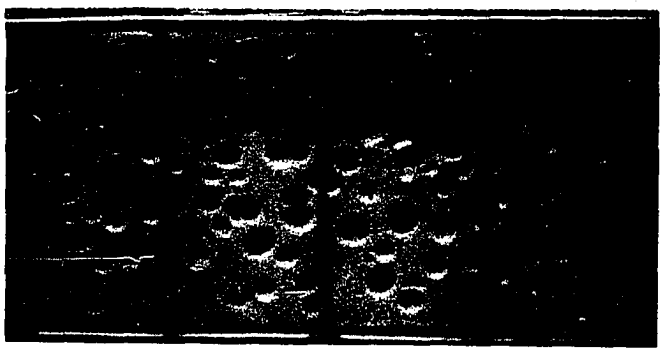


I



II

III



IV

to wrap the cheese (photos I and IV) did indicate some checks and splits, whereas, neither checks nor splits appeared in the cheese wrapped in semi- or permeable films (photos II and III).

Study of Cell-Free Extracts of Propionibacteria

The pathways by which propionibacteria utilize glucose, lactose, lactate, or other carbon sources have been extensively investigated. It is recognized that when glucose or lactate are fermented, the main products are propionic acid, acetic acid, and carbon dioxide. Since carbon dioxide, produced by these microorganisms, is the usual cause of eyes in Swiss cheese, its production may play an important role in the development of splits or checks in Swiss cheese. Splits in Swiss cheese have been related to use of certain strains of propionibacteria (93). The investigation of the role of these particular strains in the development of splits may be important in elucidating the causes of this defect.

In studying the propionibacteria it may be asked: Why do some strains possess the ability to induce splits, to produce larger quantities of carbon dioxide, and to grow at low temperatures? To attempt to answer these questions, several approaches may be considered. First, a study of the microorganisms and related environmental factors affecting growth may provide insight as to their behavior under laboratory conditions. Second, a study of their metabolism and the activity of important enzyme systems as related to environmental conditions may elucidate certain subtle differences

between the strains. The approach used to study the enzyme system of carbohydrate metabolism in propionibacteria was to select a reaction that developed activity in crude cell-free extracts. This approach was then followed by the determination of the enzyme's specific activity under various conditions of analysis. It was of primary importance to evaluate the reactions that produced carbon dioxide as an end product because of carbon dioxide's role in this study.

Several reactions involved in the metabolism of propionibacteria were investigated for determination of suitable enzyme systems. The following enzymes: glucose 6-phosphate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, lactate dehydrogenase, and pyruvate dehydrogenase were tested for activity by spectrophotometric change in absorbance. High activity was observed in cell-free extracts for malate and lactate dehydrogenases. Low activity was observed with glucose 6-phosphate and isocitrate dehydrogenases in cell-free preparations. Activity for pyruvate and succinate dehydrogenases could not be determined.

Table 12 provides information concerning the cell disruption method, pH of suspending buffer, and the total protein content of the cell-free extracts. Several techniques were investigated to determine the most efficient method for cell disruption. The greatest amount of protein was found in cell-free extracts disrupted by passage through a French pressure device and a sonic oscillation

Table 12. Preparation techniques and protein content of cell-free extracts of strains of *Propionibacterium* spp.

CFE sample no.	Strain no.	CFE treatment and disruption method	pH of suspending buffer	Total protein (mg/ml)[a]
1	59	French press-sonication	7.0	19.5
2	129	Sonication	7.0	13.5
3	59	Dialysis, lysozyme	water, 7.0	5.0
4	59	Dialysis, lysozyme	7.0	5.0
5	59	Sonic probe	7.0	3.5
6	129	French press	water, 7.0	12.5
7	129	French press-sonication	7.2	25.0
8	59	French press-sonication	7.0	16.0
9	129	French press-sonication	7.0	14.0
10	129	French press-sonication	6.0	19.3
11	129	French press-sonication	8.0	26.6
12	129	French press-sonication	9.0	22.9
13	59	French press-sonication	8.0	16.2
14	5	French press-sonication	8.0	22.8
15	83	French press-sonication	8.0	26.3
16	42	French press-sonication	8.0	24.0
17	51	French press-sonication	8.0	20.8

[a]Values are averages of triplicate determinations.

treatment. The lysozyme and the sonic probe treatments proved to be unsatisfactory for good cell disruption. The enzyme activity of several P-129 extracts decreased rapidly upon freezing. The pH of the suspending buffer was varied to determine the effect of pH on activity of the P-129 cell-free extracts. Propionibacterium arabinosum 129 cell-free samples numbered 10, 11, and 12 are identical cell preparations which were separated into three equal portions, one portion washed with pH 6 buffer, the second with pH 8 buffer, and the third with pH 9 buffer, and then suspended in their respective pH buffers. A buffer pH of 8.0 was found to be most suitable for maintaining malate dehydrogenase activity in P-129 CFE (Table 13). When lactate dehydrogenase activity at 7 C was determined, samples 10, 11, and 12 had 2200, 4510, and 4480 units of activity, respectively. A unit of lactate dehydrogenase activity is that amount of enzyme that will yield 1 µg of formazan/ mg protein. Again, results indicated that the lower pH for suspending the cell-free extracts was not suitable to maintain activity. These results indicated that the activities of the P-129 extracts were affected more severely by the lower pH than the P-59 extracts.

An experiment was performed to determine the effect of buffer pH upon activity of malate dehydrogenase. Results indicated (Figure 18) that the P-59 extract showed optimum activity at a pH of 7.5, whereas the P-129 extract had optimum activity at pH 8.5. Since the activity of malate dehydrogenase differs with respect to optimum pH in the two extracts, this data may be indicative as to why one

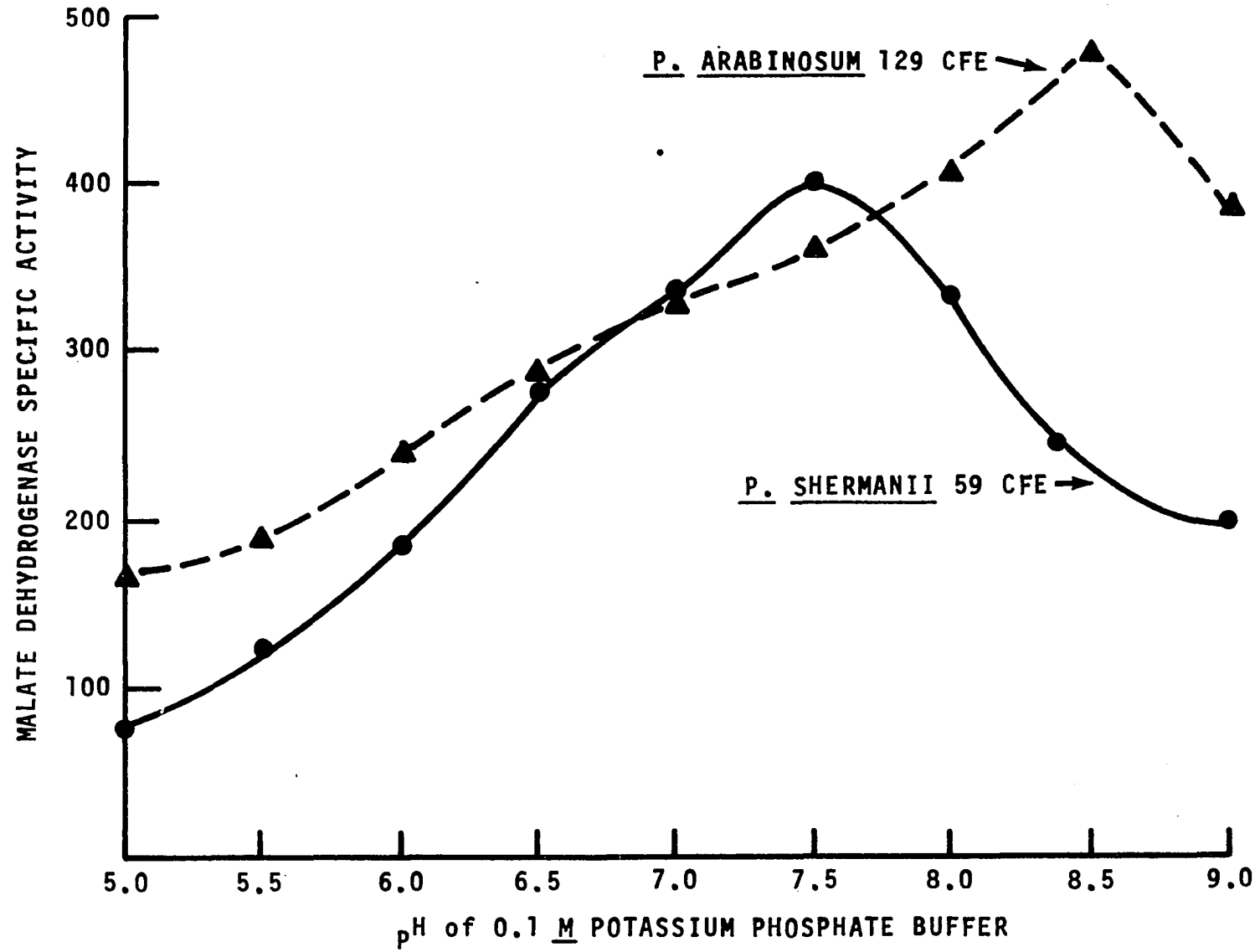
Table 13. Malate dehydrogenase activity and condition of sample of *Propionibacterium* cell-free extracts at 28 C

CFE sample no.	Strain no.	Units of activity[a]	Sample condition[b]
1	59	203	Clear, no precipitation
2	129	277	Clear, no precipitation
3	59	2	Opaque
4	59	2	Opaque
5	59	120	Clear, no precipitation
6	129	1	Precipitation
7	129	178	Clear, no precipitation
8	59	60	Clear, no precipitation
9	129	0	Precipitation
10	129	134	Slight precipitation
11	129	244	Clear, no precipitation
12	129	189	Clear, no precipitation
13	59	133	Clear, no precipitation
14	5	89	Slight precipitation
15	83	178	Clear, no precipitation
16	42	175	Clear, no precipitation
17	51	12	Precipitation

[a]Units of activity = change in absorbance at 340 nm/min/mg protein.

[b]Denotes visual observation of sample.

Figure 18. Effect of pH of 0.1 M potassium phosphate buffer on the specific activity of malate dehydrogenase


500
400
300
200
100
MALATE DEHYDROGENASE SPECIFIC ACTIVITY
P. ARABINOSUM 129 CFE
P. SHERMANII 59 CFE
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
pH of 0.1 M POTASSIUM PHOSPHATE BUFFER

extract tends to be more stable upon storage.

Nicotanamide adenine dinucleotide (NAD) oxidase was found to be active, at low levels, in the CFE preparations. It was necessary to study this reaction because of possible interference with the activity determinations of malate dehydrogenase. The thermodynamics of the reversible reaction of malate dehydrogenase greatly favor the formation of malate from oxaloacetate (75), thus when oxaloacetate and NADH are used as substrates, possible interference in obtaining valid results may occur due to the oxidase. NADH oxidase was inactivated in extracts of Bacillus cereus by heating to 60 C for 5 min (110). A similar procedure was followed to determine the time-temperature point of inactivation in extracts of propionibacteria. By heating the extract to 65 C for 5 min, no oxidase activity was observed. Malate dehydrogenase activity did remain, but at low levels. With refinements in techniques (optimum pH and substrate concentration determinations) a greater than 200-fold malate dehydrogenase activity was produced in the extracts, significantly reducing the effect caused by the presence of the NADH oxidase. This observation deleted the necessity of heating the CFE to inactivate NADH oxidase activity.

As lactic acid is oxidized to pyruvic acid, the coenzyme, NAD, is reduced. This reduction could be spectrophotometrically measured at 340 nm; however, the use of acetone-dried cells or whole cells would interfere with the method. Therefore, the dye reduction method was selected to improve the versatility of the

method. The reduced coenzyme formed in the reaction will transfer the hydrogen ions and electrons to a tetrazolium dye via the action of a mediator (phenazine-methosulfate), resulting in the reduction of the tetrazolium derivative to a red-colored formazan. The amount of formazan formed is measured by reading the absorbtion at 540 nm after 15 min.

Table 14 shows the lactate dehydrogenase activity of selected cell-free extracts of Propionibacterium spp. at various low temperatures. Since most of the P-129 cell-free extracts precipitated, resulting in low enzyme activity, CFE no. 11 was the preparation that could be considered as providing valid data. Most of the P-129 CFE were prepared using an incorrect buffer pH causing the precipitation. This problem was not corrected until the experiment of buffer pH variation was performed. Comparative values were obtained from the majority of the P-59 cell-free extracts. Cell-free extract no. 15 (P-83) repeatedly provided exceptionally high values of lactate dehydrogenase activity. The activity of cell-free extracts of several other strains of Propionibacterium spp. behaved much like the P-129 and P-59 extracts, insofar as low activity was obtained with P-5 and P-51 and relatively high activity from P-42 (extracts selected on the basis of data in Table 3). Cell-free extracts of P-5, P-51, and P-129 were extracts of strains that failed to grow at low temperatures, whereas, P-42, P-59, and P-83 strains exhibited this ability. Since P-59 CFE no. 1 and P-129 CFE no. 11 exhibited good activity at room temperature, they were

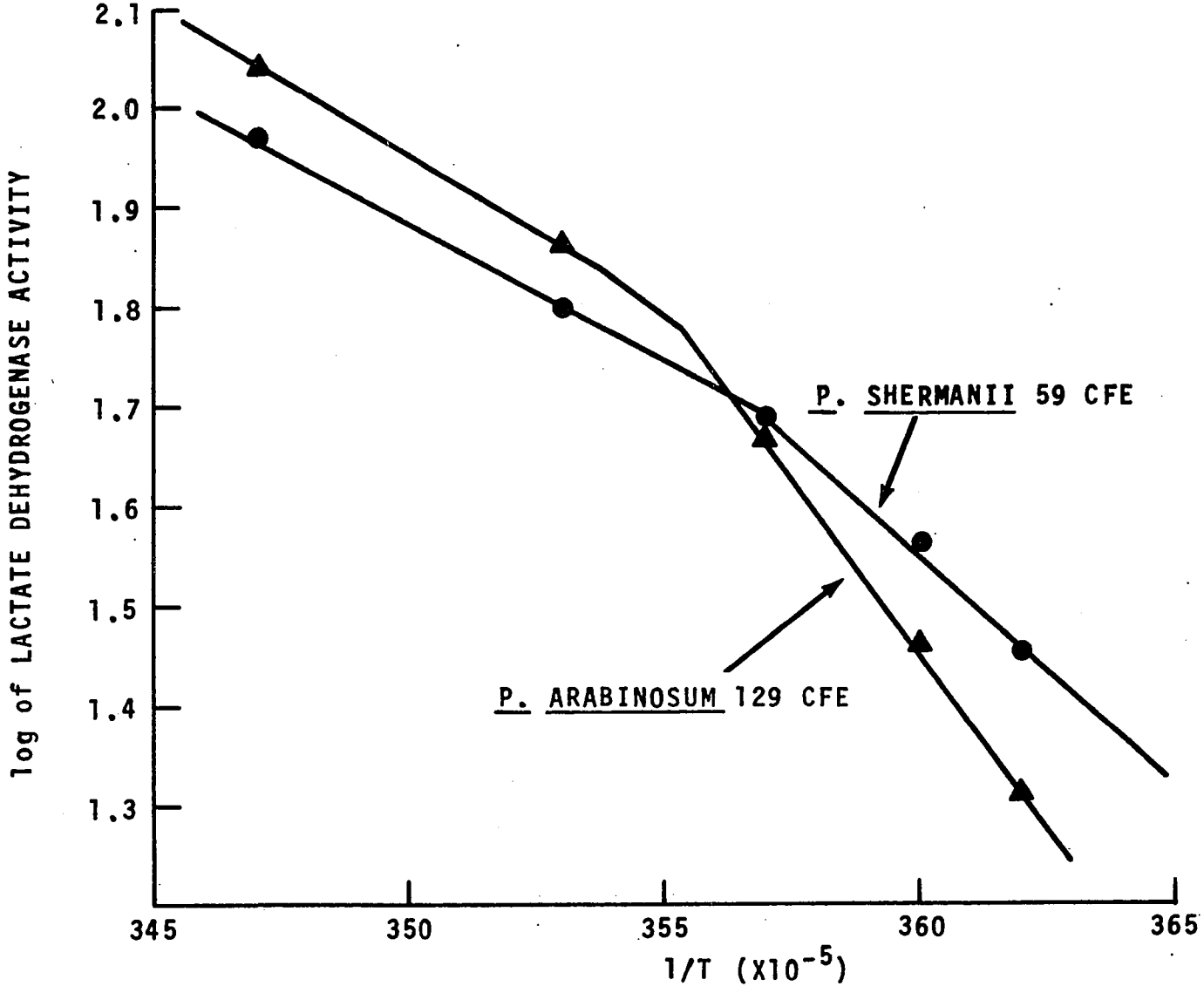
Table 14. Lactate dehydrogenase activity of cell-free extracts of Propionibacterium spp. at low temperatures

CFE sample no.	Strain no.	Units of activity[a] 5 C	10 C	15 C
1	59	3640	7280	9440
4	59	2000	4000	6000
6	129	1040	1840	3440
7	129	840	1480	2680
8	59	2810	5250	8380
9	129	1430	2400	3930
11	129	2930	7270	10800
13	59	2960	6600	8150
14	5	1320	3860	4870
15	83	7190	10270	30000
16	42	3420	6000	9130
17	51	1190	1710	3000

[a]Units of activity = µg formazan/mg protein.

selected to determine activity at 3, 5, 7, 10, and 15 C. The P-59 CFE exhibited greater lactate dehydrogenase activity than the P-129 CFE at 5 C, whereas, the reverse was true as the temperature increased to 15 C. These results were confirmed when several other cell-free extracts of similarly behaving strains were tested under similar conditions. An Arrhenius plot of temperature dependence vs the log of enzyme activity of P-59 CFE #1 and P-129 CFE #11 is depicted in Figure 19. The point at which the slope of the curves change was ambiguous due to an insufficient number of determinations. This change in slope possibly indicates that an alteration in the configuration of the enzyme occurs influencing its activity. This integer of change appears to be the rate limiting point of the enzyme. The plot indicates that the heat of activation of the enzyme is greater at high temperatures than at low temperatures. Apparently, lactate dehydrogenase is a more effective catalyst for the reaction rate at low temperatures. The interesting factor observed was that extracts obtained from Propionibacterium spp., that grew well at low temperatures, have a higher enzyme activity when determined at low temperatures than extracts obtained from strains that did not exhibit this ability. Also, the results are not prejudiced in favor of a particular extract exhibiting overall greater activity at higher temperatures. This can be observed by comparison of activity of P-129 CFE no. 11 to P-59 CFE no. 1 at 3 C and at 15 C. These results could possibly be indicative of a greater ability of certain strains of Propionibacterium spp. to metabolize and

Figure 19. Arrhenius plot of temperature dependence vs the log of specific activity of lactate dehydrogenase


2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
log of LACTATE DEHYDROGENASE ACTIVITY
P. SHERMANII 59 CFE
P. ARABINOSUM 129 CFE
345
350
355
360
365
1/T (X10^{-5})

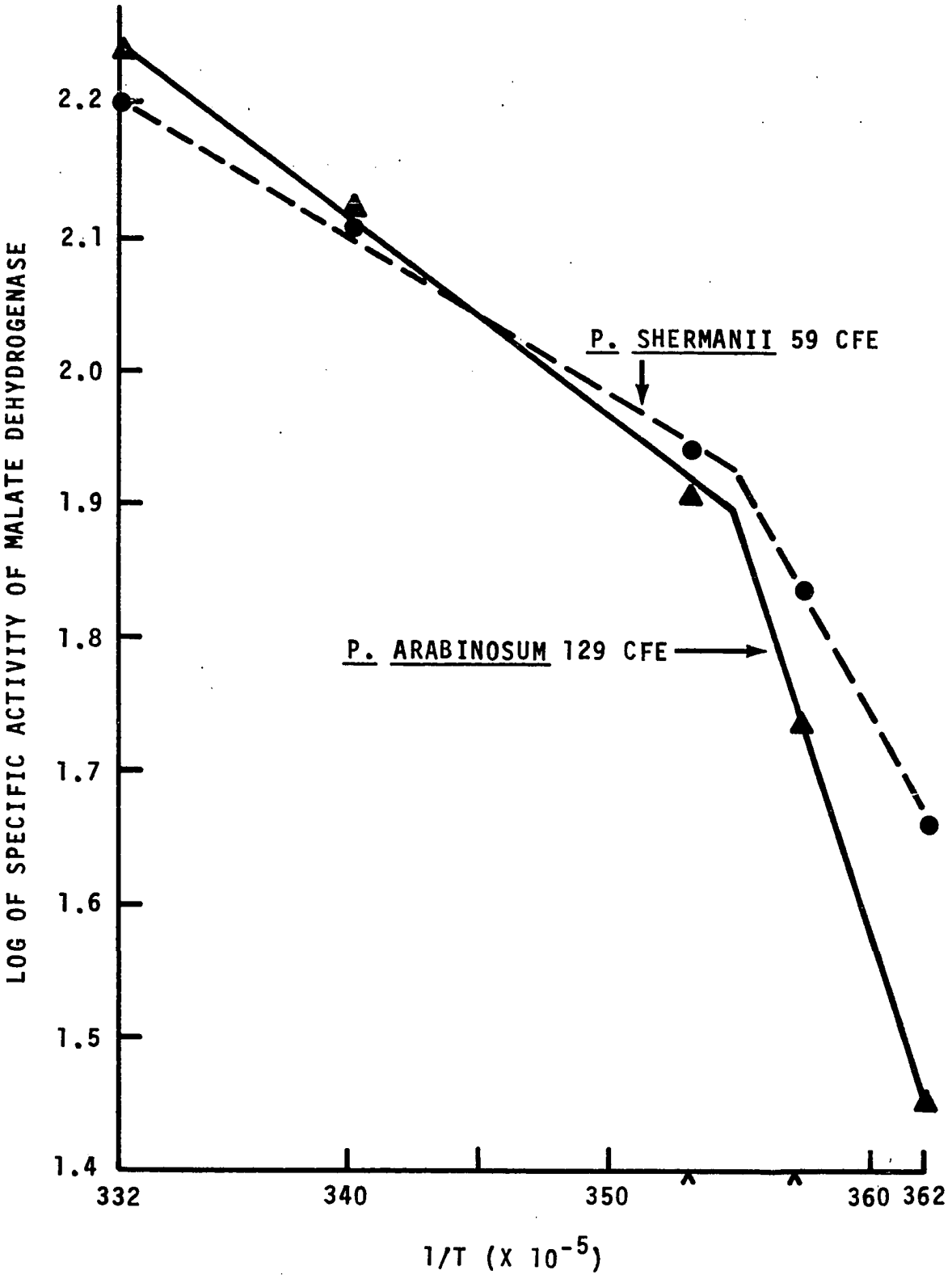
produce carbon dioxide in Swiss cheese cured at low temperatures.

Similar results were obtained when malate dehydrogenase activity was determined in extracts of P-59 and P-129 at various temperatures. Figure 20 shows an Arrhenius plot of temperature dependence vs the log of malate dehydrogenase activity of P-59 and P-129 cell-free extracts. Again, there is a possible change in the configuration of the enzyme at approximately 10 C, and also, the heat of activation is greater at higher temperatures than at low temperatures. Approximate calculations from the curve slopes indicate that the heat of activation at high temperature was 10,000 calories/mole and 4,500 calories/mole at low temperatures. Table 13 provides comparative data of malate dehydrogenase activity of all cell-free extracts prepared at 28 C and information concerning the condition of the extract used for analysis. When the CFE appeared translucent the activity was generally high, but when it appeared opaque due to precipitation, activity was low or not measurable. This criterion of sample condition aided the selection of extracts for continued analysis.

Pyruvate dehydrogenase is the enzyme responsible for the production of carbon dioxide in propionibacteria (1). The study of this enzyme may provide insight as to the ability of certain strains of Propionibacterium spp. to grow and metabolize at low temperatures.

The study of pyruvate dehydrogenase was initiated to establish activity of this enzyme or enzyme complex at various temperatures. Reed and Willms (101), in studying the pyruvate dehydrogenase

Figure 20. Arrhenius plot of temperature dependence vs the log of specific activity of malate dehydrogenase


LOG OF SPECIFIC ACTIVITY OF MALATE DEHYDROGENASE
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
1.4
P. SHERMANII 59 CFE
P. ARABINOSUM 129 CFE
332
340
350
360
362
1/T (X 10⁻⁵)

complex in E. coli, stated that a spectrophotometric assay based on measurement of the rate of formation of NADH can be applied after some purification of the enzyme complex. They found preparations had to be free of NADH oxidase and lactate dehydrogenase before activity could be spectrophotometrically measured. Since no activity was observed in Propionibacterium cell-free extracts, similar inhibitions may be present.

The dismutation assay for the measurement of acetyl phosphate will indicate activity at all levels of purity in E. coli CFE (101). This method was attempted without the addition of phosphotransacetylase and activity could not be observed in Propionibacterium cell-free extracts. To attempt to determine if an incorrect procedure was being followed and if the phosphotransacetylase addition affected the results, an E. coli B cell-free extract was freshly prepared and tested by the method. Also, CFE of E. coli K-12 and E. coli W were obtained from the Department of Bacteriology, Iowa State University, and were tested. Escherichia coli B and E. coli W cell-free extracts exhibited the presence of a slight amount of acetyl phosphate being produced. No acetyl phosphate was produced in the E. coli K-12 CFE. These results, though inconclusive, indicated that a correct procedure was being followed and that the addition of phosphotransacetylase was important to obtain activity in E. coli CFE. Allen et al. (1) were able to show that phosphotransacetylase existed in CFE of P. shermanii. Since the presence of phosphotransacetylase in Propionibacterium has been shown to

exist, it should not be necessary to add this enzyme to samples being tested. This supposition, of course, does not account for interference by inhibitors, inactivation by environmental conditions, feed-back inhibition by intermediates and products, and/or the many other factors influencing activity.

Manometric determinations of carbon dioxide production by Propionibacterium CFE were negative. By the procedure employed (71), no carbon dioxide was liberated from P-59, P-83, and P-129 CFE samples tested. Manometric measurements of carbon dioxide from E. coli B whole cells and cell-free extracts were negative, suggesting the procedure employed was incorrect, as determined in this laboratory.

A final attempt to obtain pyruvate dehydrogenase activity was tried by substituting substrates as employed for the determination of lactate dehydrogenase. When acetyl-CoA was added to the mixture containing buffer, INT, pyruvate, but no CFE or other substrates and cofactors, a color complex was formed. The formation of a color complex without the addition of CFE negated the use of this assay.

To determine if an alternate pathway for carbon dioxide formation, different from that pathway described by Allen et al. (1), existed in propionibacteria, several other substrates were substituted for pyruvate. When phosphoenolpyruvate (PEP) and dihydroxyacetone phosphate (DHP) were added to the sample mixture for manometric measurement of carbon dioxide liberation and for acetyl phosphate production, no activity was determined.

SUMMARY AND CONCLUSIONS

To study the role of propionibacteria in the development of splits in Swiss cheese two general approaches were followed: first, to study the role of propionibacteria in utilization of lactate and sugar, degree of proteolysis, carbon dioxide production, and their growth in Swiss cheese; second, to study their metabolism, growth at low temperatures, and carbon dioxide production in pure culture. Also, a specific approach was followed to determine more subtle differences in metabolic capabilities between strains of Propionibacterium spp.

A comparison of characters between two different propionibacteria was first made. Selection of these two propionibacteria was based on previous observations of split and nonsplit Swiss cheese resulting from the use of particular strains of Propionibacterium spp. in cheese manufacture. Also, strain selection was based on ability or lack of ability to grow at low temperatures (3.8 C). In addition, several other strains were tested under similar conditions to provide colloborative data. The two major strains selected were Propionibacterium shermanii 59, a splitter with the ability to grow at low temperatures, and Propionibacterium arabinosum 129, a nonsplitter lacking the ability to grow at low temperatures.

The following general observations were made:

1. Strains of Propionibacterium spp. have the ability to grow

at temperatures as low as 3.8 C in Sodium lactate broth. These strains generally belonged to species of P. shermanii and P. freudenreichii. Propionibacterium shermanii strains 47, 59, and 83, having the ability to grow at low temperatures, and P. arabinosum 129 and P. pentosaceum 5, both not able to grow at low temperatures, were the strains most frequently utilized in this investigation.

2. Strains of Propionibacterium spp., selected on the basis of growth at low temperatures, were able to produce carbon dioxide at 3.8 (0.4 ml) and 10 C (1.5 ml) and approximately six times more carbon dioxide at 21 C than strains lacking the ability to grow at low temperatures in Sodium lactate broth.

3. Propionibacteria that grew at low temperatures produced a higher incidence of checks and splits (10 of 13 lots split at 6 months of age) in Swiss cheese than those strains that lacked the ability to grow at low temperatures (2 of 10 lots split at 6 months of age).

4. The rate of lactic acid utilization, carbon dioxide production, and increases in cell numbers generally correlated with one another in relationship to the use of particular strains of Propionibacterium spp. in cheese manufacture.

5. No correlation of the degree of proteolysis, moisture content, and the disappearance of sugars could be made with the incidence of splits and the Propionibacterium strain used in the manufacture of Swiss cheese.

6. Glucose, galactose, and lactose disappeared rapidly from the cheese. Glucose was found in Swiss cheese during the pressing period but disappeared 24 hr after manufacture. Galactose concentrations were 10 times (14.8 mg/g) greater than those for glucose during the pressing period. Low galactose concentrations (0.3 mg/g) could be detected in the cheese 24 hr after manufacture but disappeared within 48 hr.

7. The type of cheese wrapping film used will affect the incidence of splits and checks in Swiss cheese. Cheeses wrapped in gas permeable films have a decreased disposition toward splitting as compared with cheeses wrapped in impermeable films.

The following specific observations were made:

1. Propionibacterium shermanii 59, when used in the manufacture of Swiss cheese, had a greater tendency to induce splitting than P. arabinosum 129.

2. Propionibacterium shermanii 47, 59, and 83 were concluded to be cheese splitters and P. arabinosum 129 and P. pentosaceum 5 were nonsplitters.

3. Propionibacterium shermanii 59 grew faster and to greater total numbers in pure culture and in Swiss cheese than did P. arabinosum 129. Strain P-59 increased numerically to $>$ 1 billion/g cheese, whereas, P-129 increased to approximately 500 million/g cheese.

4. Propionibacterium shermanii 59 produced greater than a double rate of carbon dioxide during the hot room cure of Swiss

cheese and a higher level of continuing carbon dioxide production during the cold room cure as compared with P. arabinosum 129. Propionibacterium shermanii 47 and 83 produced greater than two times the rate of carbon dioxide during the hot room cure than did P-59. Cheese made with P-47 and P-83 split. Propionibacterium pentosaceum 5 produced amounts of carbon dioxide equivalent to that in Swiss cheese made with the P-129 strain, and, as with P-129, the cheese did not split.

For a strain of Propionibacterium to have the ability to produce splits in Swiss cheese and to be able to grow and produce carbon dioxide at low temperatures, the microorganism must possess metabolic capabilities that are unusual or, at least, different from those in strains lacking these abilities. Characters unusual to the particular strain may result from the effect of temperature and pH on the activity of the metabolic enzymes. The following specific observations of these characters were made:

1. Cell-free extracts of P-129 precipitated when prepared and suspended in pH 7.0 potassium phosphate buffer. When the pH was increased to 8.0 the precipitation was eliminated.

2. Propionibacterium arabinosum 129 cell-free extracts had the greatest malate dehydrogenase specific activity when analyses were performed at pH 8.5. Propionibacterium shermanii 59 cell-free extracts had the greatest specific activity when analyses were performed at pH 7.5. These results indicate a distinct difference between the extracts of the two species. Also, the results

suggested why, in cheese with an approximate pH of 5.5, P. arabinosum 129 cells had a slower growth rate and a decreased ability to produce carbon dioxide as compared with P. shermanii 59 cells.

3. Malate and lactate dehydrogenase specific activity could be demonstrated in all cell-free extracts of strains of Propionibacterium spp.

4. Cell-free extracts of P-129 demonstrated equivalent, if not greater, lactate and malate dehydrogenase specific activity at 15 C or greater than did P-59 CFE. As the temperature was decreased from 10 to 3 C, P-59 CFE exhibited greater specific activity than did P-129 CFE. These results indicated that P-59 CFE was capable of greater enzymic activity and had a larger metabolic capacity to operate at lower temperatures than did P-129 CFE.

5. Since carbon dioxide played an important role in this study it was important to investigate the enzymic reactions by which carbon dioxide is formed by propionibacteria. It has been suggested that carbon dioxide is formed by the decarboxylation of of pyruvate and that the pyruvate dehydrogenase complex is responsible for the reaction. Determination of the effect of temperature upon the specific activity of pyruvate dehydrogenase may provide insight as to the metabolic capabilities for carbon dioxide production by a strain of Propionibacterium. Results of analyses showed that pyruvate dehydrogenase specific activity

could not be demonstrated in Propionibacterium CFE by spectrophotometry, by manometry, by the manometric dismutation assay, or by the dismutation assay for acetyl phosphate formation. It was hypothesized that since activity for pyruvate dehydrogenase could not be demonstrated, interference and/or inhibition of the enzyme reaction occurred in the cell-free extracts. Also, the proposed reaction mechanism or the proposed substrate required may not be correct.

6. An alternate pathway for carbon dioxide formation may exist in propionibacteria. The conversion of pyruvate to acetate and carbon dioxide has been the hypothesized route of formation. When phosphoenolpyruvate and/or dihydroxyacetone phosphate were substituted for pyruvate as the substrate(s), no activity was demonstrated. These results indicated that these compounds do not serve as substrates for an alternate pathway for carbon dioxide formation under the conditions analyzed.

Many factors influence the disposition toward splitting. For instance, it is generally considered that large cheese blocks may be more likely to split than smaller blocks, that too-rapid acid development in curd in the press, and that overdeveloped and overset cheese will increase this tendency.

It is now evident that the split defect does not necessarily reflect an unusual degree of proteolysis in split cheese, but does show the effect of continuing gas production, increased gas retention, greater microbial growth, and enhanced substrate utilization as the cheese ages. From these results it can be seen that

there is no single factor or single strain of propionibacteria that may be responsible, but that certain strains have a greater tendency to produce split cheese. Not tested in this work but certainly of major importance is the fact that microorganisms other than Propionibacterium also may grow, metabolize, and produce carbon dioxide in Swiss cheese. In any event, because of the fact that older cheeses split more readily, the cheese must be protected from temperature fluctuations as it ages. Heat shocking, the process whereby the temperature of the cheese is raised and then is lowered, will cause the carbon dioxide to become less soluble, thus expansion will occur. If the cheese body is sufficiently inelastic, splits are likely to occur, especially since Swiss cheese is essentially supersaturated with carbon dioxide at temperatures below 10 C. The data presented on carbon dioxide production, lactate disappearance, growth, enzymic differences, and the tendency toward splitting indicated that P-59 and other strains that grow at low temperatures differed significantly in their behavior in cheese, in pure culture, and in their cell-free extracts than did P-129 and other strains lacking the ability to grow at low temperatures.

The wrapper plays an important role in the release of carbon dioxide from the supersaturated cheese. The wrapper closely adheres to the surface of the cheese, serving as an artificial rind and as a barrier for carbon dioxide diffusion. If the wrapper is sufficiently impermeable, the carbon dioxide will not be allowed

to escape, thus increasing the pressure within the cheese. It is recognized that other microorganisms could conceivably produce gases other than carbon dioxide which could split cheese, but these factors were not investigated in this study.

The results on enzymic studies indicated that extracts from strains of Propionibacterium spp., suspected as splitters, had greater metabolic capabilities at lower pH and lower temperatures than did extracts of strains considered to be nonsplitters. These results support the hypothesis that certain propionibacteria are capable of causing splits in Swiss cheese.

LITERATURE CITED

1. Allen, S. H. G., R. W. Kellermeyer, R. L. Stjernholm, and H. G. Wood. 1964. Purification and properties of enzymes involved in the propionic acid fermentation. J. Bacteriol., 87: 171-187.

2. Andres, J., and O. Bolliger. 1963. Comparative determination of pH and moisture content in different places of young Emmental cheese. Schweiz. Milchztg. 89, Wiss. Beil., 88: 701-706. (Dairy Sci. Abstr., 25: 251).

3. Antila, M. 1954. Über die Propionsäurebakterien im Emmentaler Käse. Keijerit. Aikakausk., 16: 1-132.

4. Antila, M., and M. Hietaranta. 1953. Wachstumshemmung der Propionsäurebakterien durch Propionat. Meijerit. Aikakausk., 15: 3-10.

5. Antila, M., and M. Hietaranta. 1953. Wachstums- und Gärungshemmung der Propionsäurebakterien durch Laktat. Meijerit. Aikakausk., 15: 11-19.

6. Babel, F. J., and B. W. Hammer. 1939. Bacteriology of cheese. IV. Factors affecting the ripening of Swiss-type cheese made from pasteurized milk. Iowa State Coll. Agr. Exp. Sta. Bull. 264. 48 pp.

7. Barker, H. A., and F. Lipmann. 1944. On lactic acid metabolism in propionic acid bacteria and the problem of oxido-reduction in the system's fatty-hydroxyketo acid. Arch. Biochem., 4: 361-370.

8. Bijok, F., and M. Domanska-Strzatkowska. 1970. Studies on starter activation and on the addition of water to the curd after cutting during the manufacture of Emmental cheese. XVIII Int. Dairy Congr., IE: 368.

9. Bolliger, O. 1967. Heat treatment of milk or curd as a means of reducing eye formation in Emmental cheese. Schweiz. Milchztg. 93, Wiss. Beil., 112: 920-922. (Dairy Sci. Abstr., 29: 4305).

10. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1967. Bergey's manual of determinative bacteriology. 7th ed. The Williams and Wilkins Co., Baltimore, Md. 1094 p.

11. Budslawski, J., and K. Pogorzelski. 1964. Fermentation of lactose in different varieties of cheese (in French, English summary). Lait, 44: 496-505. (Dairy Sci. Abstr., 27: 247).

12. Burkhalter, G. 1956. Einfluss von nichtrostendem Stahl im Vergleich zu Kupfer als Käsekesselmaterial bei der Fabrikation von Emmentalerkäse. Schweiz. Milchztg. Wiss. Beil., 41: 321-327.

13. Burkhalter, G., E. Flückiger, and P. Schilt. 1957. Die oberflächenbehandlung beim Emmentaler mit Käsedeckmitteln. Schweiz. Milchztg., 83, Wiss. Beil., 49: 385-392.

14. Burkhalter, G., and P. Obrist. 1957. Praktische Versuche über die Oberflächenbehandlung von Emmentalerkäse mit Foodplast. Schweiz. Milchztg., 83: 332.

15. Burkey, L. A., G. P. Sanders, and K. J. Matheson. 1935. The bacteriology of Swiss cheese. IV. Effect of temperature upon bacterial activity and drainage in the press. J. Dairy Sci., 18: 719-731.

16. Burtscher, H. 1937. Die Pasteurisierung der Emmentaler-Käsereimilch. XI Milchwirtschaftlicher Weltkongress, 2: 179-182.

17. Clark, W. M. 1912. A study of the gasses of Emmental cheese. U. S. Dep. Agr., Bur. Anim. Ind. Bull. 151.

18. Clark, W. M. 1917. On the formation of "eyes" in Emmental cheese. J. Dairy Sci., 1: 91-113.

19. Deane, D. D., and F. D. Cohenour. 1961. Rindless, Swiss-type cheese. I. Method of manufacture in block form. J. Dairy Sci., 44: 451-456.

20. Decker, J. W. 1905. Cheese making. Berlin Printing Co., Columbus, Ohio. p. 141.

21. Demeter, K. J., A. Janoschek, and E. Gunther. 1953. Weiteres über die Bakteriologie der Emmentalerkäse-Bereitung und-Reitung. Milchwiss., 8: 420-426.

22. Doležálek, J., B. Plachý, and E. Hromadniková. 1962. Study of propionic acid bacteria and their effect on quality of cheese of the Emmental type. Sborn. Vysoke Skoly Chem.-tech. v Praze, Potravin Tech., 6: 93-106. (Dairy Sci. Abstr., 26: 20).

23. Dorner, W., O. Langhard, W. Mosimann, und P. Ritter. 1938. Käsereiversuche mit Propionsäurebaktereinkulturen (in German, English summary). Schweiz. Milchztg., 64: 553-554.

24. Dorner, W., und P. Ritter. 1938. Untersuchungen über Verfahren sur Betriebskontrolle in der Emmentalerkäseri. Landw. Jahrb. Schweiz., 52: 339-366.

25. Dorner, W., and J. Sähli. 1934. Extrail des rapports de la fromagerie d'expérimentation de Treyvauz (in French, English summary). Lanw. Jahrb. Schweiz., 48: 769-794.

26. Dürichen, K., R. Heiss, and K. Becker. 1970. Gas concentration changes in Emmental cheese packaged in plastic film and their significance in retention of cheese quality. Dte Molk.-Stg., 91: 385-391. (Dairy Sci. Abstr., 32: 472).

27. Emilsson, E., and G. Sjöström. 1947. The preparation of sterile experimental cheese on the laboratory scale, and some experiments on the ability of some bacteria to break down citric acid in cheese. Svenska Mejeritidn., 39: 467-472. (Dairy Sci. Abstr., 10: 199).

28. Fitz, A. 1878. Über Spaltpilzgährungen. Ber., 11: 1890; 1879, 12: 474; 1880, 13: 1309; 1882, 15: 867; 1883, 16: 844; 1884, 17: 1188.

29. Flückiger, E., and H. Hostettler. 1957. Beitrag zur Nachgärungsdisposition des Emmentalerkäses. Schweiz. Milchztg., 89, Wiss. Beil., 95: 758-760.

30. Flückiger, E., and O. Reber. 1963. Estimation of carbon dioxide content in Emmental cheese. Schweiz. Milchztg., 89, Wiss. Beil., 95: 758-760. (Dairy Sci. Abstr., 26: 94).

31. Flückiger, E., and G. Roy. 1961. Study of the prepackaging of Emmental cheese. Schweiz. Milchztg., 87, Wiss. Beil., 80: 637-644. (Dairy Sci. Abstr., 27: 480).

32. Flückiger, E., and H. Zürcher. 1958. Über die Nächgarungsdisposition von in Kupfer- oder Stahlkesseln hergestellten Emmentalerkäsen. Schweiz. Milchzeit., Wiss. Beil., 59: 465-471. (Dairy Sci. Abstr., 21: 398).

33. Frazier, W. C., L. A. Burkey, A. J. Boyer, G. P. Sanders, and K. J. Matheson. 1935. The bacteriology of Swiss cheese. II. Bacteriology of the cheese in the press. J. Dairy Sci., 18: 373-387.

34. Frazier, W. C., W. T. Johnson, Jr., F. R. Evans, and G. A. Ramsdell. 1935. The bacteriology of Swiss cheese. III. The relation of acidity of starters and of pH of the interior of Swiss cheese to quality of cheeses. J. Dairy Sci., 18: 503-510.

35. Frazier, W. C., G. P. Sanders, A. J. Boyer, and H. F. Long. 1934. The bacteriology of Swiss cheese. I. Growth and activity of bacteria during manufacturing processes in the Swiss cheese kettle. J. Bacteriol., 27: 539-549.

36. Frazier, W. C., and H. U. Wing. 1931. Studies on the eye-forming organism of Swiss cheese. J. Bacteriol., 21: 39-40. (Abstr.)

37. Frazier, W. C., and H. U. Wing. 1932. Bacterium acidi-propionici and other lactate-fermenting bacteria of Swiss cheese. J. Bacteriol., 23: 60-61. (Abstr.)

38. Futschik, J. 1961. The ripening of Austrian "Stangen" cheese in film. Milchwiss. Berichte, Wolfpassing, 11: 135-150. (Dairy Sci. Abstr., 25: 59).

39. Gardet, F. 1967. A case of slit eyes (in Emmental cheese). Techq. Lait., 575: 37-38. (Dairy Sci. Abstr., 30: 487).

40. Gardet, F. 1968. Slit eyes in Emmental cheese. Techq. Lait. 598: 10-11.

41. Gomori, G. 1955. Preparation of buffers for use in enzyme studies, vol. 1, p. 138-146. In S. P. Colowick and N. O. Kaplan (ed.) Methods in Enzymology. Academic Press, Inc., New York, New York.

42. Goss, E. F., V. H. Neilsen, and M. Mortensen. 1938. Iowa Swiss-type cheese. Iowa State Coll. Agr. Exp. Sta. Bull. 378.

43. Hammond, E. G., and G. W. Reinbold. 1965. Study of the split defect in Swiss cheese. J. Dairy Sci., 48: 739-740.

44. Hammond, E. G., R. G. Seals, and G. W. Reinbold. 1966. Determination of proteolysis by dye binding. J. Dairy Sci., 49: 504-506.

45. Hanson, F. E., D. W. Spicer, and W. V. Price. 1938. Observations on the splitting of Brick cheese. J. Dairy Sci., 21: 433-436.

46. Harper, W. J., and H. E. Randolph. 1960. Lactic acid in Cottage cheese. Amer. Milk Rev., 22: 43-46.

47. Hettinga, D. H., A. H. Miah, E. G. Hammond, and G. W. Reinbold. 1970. Sensitive enzymatic method for determination of glucose, galactose, and lactose in Cheddar cheese. J. Dairy Sci., 53: 1377-1380.

48. Hettinga, D. H., and G. W. Reinbold. 1972. The propionic-acid bacteria—A review. I. Growth. J. Milk Food Technol., (in press).

49. Hettinga, D. H., and G. W. Reinbold. 1972. The propionic-acid bacteria—A review. II. Metabolism. J. Milk Food Technol., (in press).

50. Hettinga, D. H., and G. W. Reinbold. 1972. The propionic-acid bacteria—A review. III. Miscellaneous metabolic activities. J. Milk Food Technol., (in press).

51. Hettinga, D. H., E. R. Vedamuthu, and G. W. Reinbold. 1968. Pouch method for isolating and enumerating propionibacteria. J. Dairy Sci., 51: 1707-1709.

52. Hettinga, D. H., E. R. Vedamuthu, G. W. Reinbold, and E. G. Hammond. 1969. Studies on the split defect in Swiss cheese. J. Dairy Sci., 52: 880.

53. Hietaranta, M., and M. Antila. 1953. The influence of biological nutrients on the growth of propionic acid bacteria in milk. XIII Int. Dairy Congr., 3: 1428-1431.

54. Holmes, B., W. E. Sandine, and P. R. Elliker. 1968. Some factors influencing carbon dioxide production by Leuconostoc citrovorum. Appl. Microbiol., 16: 56-61.

55. Hunter, J. E. 1959. Gas production by associated Swiss cheese bacteria. Diss. Abstr., 20: 1962-1963.

56. Hunter, J. E., and W. C. Frazier. 1961. Gas production by associated Swiss cheese bacteria. J. Dairy Sci., 44: 2176-2186.

57. Jager, H. 1960. Casein breakdown in Emmental cheese. Milchwiss. Ber., Wolfpassing, 10: 1-10. (Dairy Sci. Abstr., 23: 143).

58. Kiermeier, F., A. Mayr, and J. Hanusch. 1968. Zur Entstehung flüchtiger Fettsäuren bei der Propionsäure- und Buttersäuregarung im Emmentalerkäse. I. Einfluss der Propionsäuregarung. Z. Lebensm. u.-Forsch., 136: 193-203.

59. Kiermeier, F., and G. Weiss. 1970. Influence of copper on acid formation in cheese. XVIII Int. Dairy Congr., 1E: 374.

60. Kiuru, V. J. T. 1948. The effect of oxidation-reduction potential on the rate of development of propionic acid fermentation. Meijerit. Aikakausk., 10: 67-74. (Dairy Sci. Abstr., 10: 201).

61. Kiuru, V. J. T. 1949. Factors affecting the velocity of propionic acid fermentation. XII Int. Dairy Congr., 2: 499-508.

62. Kiuru, V., and H. Uotila. 1967. Effect of the rind on keeping quality of Emmental cheese. Karjantuote, 50: 385. (Dairy Sci. Abstr., 30: 486).

63. Klimovskii, I., and K. Alekseeva. 1964. Effect of lactic acid bacteria and their metabolic products on the growth of propionic acid bacteria. Mol. Prom., 25: 15-17. (Dairy Sci. Abstr., 26: 1982).

64. Klimouskii, I., K. Alekseeva, and K. Cheklova. 1965. Propionic acid bacteria and their effect on the biochemical processes and quality of Soviet cheese. Mol. Prom., 26: 16-18. (Dairy Sci. Abstr., 27: 1821).

65. Knĕz, V. 1962. Effect of the size of curd grains on the numbers of eyes in Emmental cheese. Prumysl Potravin, 13: 243-247. (Dairy Sci. Abstr., 24: 2458).

66. Koestler, G. 1933. Zur Kenntnis der chemischen Grundlagen der Gärungen im Emmentalerkäse. Landw. Jahrb. Schweiz., 47: 156-202.

67. Koestler, G. 1933. Betrachtungen über den wahren Säuregrad (Wasserstoffionenkonzentration) unserer Hartkäse und dessen Beziehungen zur Teigbeschaffenheit. Landw. Jahrb. Schweiz., 47: 1121-1146.

68. Koestler, G. 1940. Untersuchungen über die Elastizität des Emmentalerkäseteiges. Landw. Jahrb. Schweiz. 54: 127-141.

69. Koestler, G. 1947. Über die Ursachen verschiedener Teigbildung beim Emmentalerkäse. Schweiz. Milchztg., 72: 66-68; 73: 51-52.

70. Koestler, G. 1953. Curd particles and their significance for the body properties of Emmental cheese. Schweiz. Milchztg. 79: 247. (Dairy Sci. Abstr., 15: 700).

71. Korkes, S. 1955. Coenzyme A-linked pyruvic oxidase (bacterial), vol. 1, p. 490-495. In S. P. Colowick and N. O. Kaplan (ed.) Methods in Enzymology. Academic Press, Inc., New York, New York.

72. Kurman, J. 1960. Neure Beobachtungen über die Aufbewahrung und Behandlung der Propionsäurebakterienkultur. Schweiz. Milchztg., 43: 1.

73. Kurmann, J. A. 1967. Studies on the control of propionic fermentation in Gruyére cheese made from raw milk. I. Lack of eyes, its causes and remedies. Schweiz. Milchztg., 93: 183-185. (Dairy Sci. Abstr., 29: 407).

74. Kurtz, F. E., J. E. Hupfer, E. A. Corbin, R. E. Hargrove, and H. E. Walter. 1959. Interrelationships between pH, populations of Propionibacterium shermanii, levels of free fatty acids, and the flavor ratings of Swiss cheese. J. Dairy Sci., 42: 1008-1019.

75. Lehninger, A. L. 1970. Biochemistry. Worth Publishers, Inc., New York, N.Y. p. 353.

76. Ling, E. R. 1951. The determination of lactic acid in milk. J. Sci. Agr., 2: 279-288.

77. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 193: 265-275.

78. Malik, A. C., G. W. Reinbold, and E. R. Vedamuthu. 1968. An evaluation of the taxonomy of Propionibacterium. Can. J. Microbiol., 14: 1185-1191.

79. Maurer, L. 1971. Effect of copper on certain Swiss cheese characteristics. M.S. Thesis. Iowa State Univ. Libr., Ames, Iowa.

80. Molinari, R., and F. J. S. Lara. 1960. The lactic dehydrogenase of Propionibacterium pentosaceum. Biochem. J., 75: 57-65.

81. Mueller, H. F., W. O. Nelson, and W. A. Wood. 1952. The effect of copper upon the activity of bacteria associated with Swiss cheese manufacture. J. Dairy Sci., 35: 929-936.

82. Neronova, N. M., and S. I. Ibragimova. 1969. Formation of volatile acids and vitamin B_{12} by Propionibacterium shermanii growing in continuous culture with an excess of lactate. Mikrobiologiya, 38: 420-424.

83. Neronova, N. M., S. I. Ibragimova, and N. D. Ierusalimshii. 1967. Effect of propionate concentration of specific growth rate of Propionibacterium shermanii. Mikrobiologiya, 36: 404-409.

84. Nieuwenhof, E. F. J., J. Stadhouders, and G. Hup. 1969. Stimulating effect of lactobacilli on growth of propionibacteria in cheese. Neth. Milk Dairy J., 23: 287-289.

85. Oehen, V. 1967. Is there still a risk of "sour Glassler" cheese with the present milk quality? Schweiz. Milchztg. 93: 551. (Dairy Sci. Abstr., 30: 369).

86. Oehen, V., and J. Andres. 1965. The effect of size of the inoculum of propionic acid bacteria on the eye formation in Emmental cheese. Schweiz. Milchztg., 91, Wiss. Beil., 104: 829-836. (Dairy Sci. Abstr., 27: 386).

87. Oehen, V., and O. Bolliger. 1963. Effect of cold storage before warm curing on the quality of Emmental cheese. Schweiz. Milchztg., 89, Wiss. Beil., 90: 715-722. (Dairy Sci. Abstr., 26: 368).

88. Oehen, V., W. Ritter, and O. Bolliger. 1960. Studies on the effect of the size of inoculum of propionic acid culture on the formation of eyes in cheese of high initial pH. Schweiz. Milchztg., 86, Wiss. Beil., 71: 561-571. (Dairy Sci. Abstr., 23: 58).

89. Oehen, V., W. Ritter, O. Bolliger, K. Sähli, and G. Tomka. 1958. The effect of the inoculum size of propionic acid culture on eye formation in Emmental cheese. Schweiz. Milchztg., 84, Wiss. Beil., 63: 489-502. (Dairy Sci. Abstr., 21: 2603).

90. Olšanský, Č., R. Minařík, and L. Rojíková. 1966. Effect of salt content on the quality of Emmental cheese. Prumysl. Potravin, 17: 544-546. (Dairy Sci. Abstr., 29: 907).

91. Orla-Jensen, S. 1898. Studien uber die Lochbildung in den Emmentaler Kasen. Zentralbl. Bacteriol. Parasitenk., 4: 217-222, 265-275, 325-331.

92. Orla-Jensen, S. 1931. Dairy Bacteriology. 2nd ed. P. Blakiston's Son and Co., Inc., Philadelphia, Penn.

93. Park, H. S., G. W. Reinbold, and E. G. Hammond. 1967. Role of propionibacteria in split defect of Swiss cheese. J. Dairy Sci., 50: 820-823.

94. Park, H. S., G. W. Reinbold, E. G. Hammond, and W. S. Clark. 1967. Growth of propionibacteria at low temperatures. J. Dairy Sci., 50: 589-591.

95. Parmelee, C. E. 1967. Applications of simple procedures for detecting gas production by lactic cultures. (Abstr.) J. Dairy Sci., 50: 953.

96. Peltola, E. 1940. Effect of salt on bacteria of importance in Emmental cheese-making. Meijerit. Aikakausk., 2: 11-21. (Dairy Sci. Abstr., 11: 229).

97. Peters, I. I., and A. V. Moore. 1958. Studies related to the manufacture of Swiss-type cheese. I. Use of homogenized milk. J. Dairy Sci., 41: 70-73.

98. Peters, I. I., and A. V. Moore. 1958. Studies related to the manufacture of Swiss-type cheese. II. Factors influencing ripening. J. Dairy Sci., 41: 74-78.

99. Pulay, G. 1958. Breakdown of lactose in cheese. I. Lactose breakdown in Emmental cheese. II. Lactose breakdown in Pannonia cheese. Tejipar. Kutat. Kozlem., 1: 1-10. (Dairy Sci. Abstr., 23: 394).

100. Pulay, G., S. N. Toth, and A. R. Bakos. 1959. Importance of iron in the metabolism of some microorganisms of importance to the dairy industry. XV Int. Dairy Congr., 2: 775-781.

101. Reed, L. J., and C. R. Willms. 1966. Purification and resolution of the pyruvate dehydrogenase complex in Escherichia coli. Vol. IX, p. 247-265. In: S. P. Colowick and N. O. Kaplan (ed.) Methods in enzymology.

102. Reinbold, G. W. 1972. Swiss cheese varieties. Vol. V. Chas. Pfizer and Co., Inc., New York, N.Y. (in press).

103. Reinbold, G. W., R. V. Hussong, and J. B. Stine. 1958. Distribution of propionibacteria in Swiss cheese. J. Dairy Sci., 41: 606-610.

104. Reiner, M., G. W. Scott-Blair, and G. Mocquot. 1949. The mechanism of the formation of slits in Gruyère cheese. Lait, 29: 351-357.

105. Ritter, P., and H. Schwab. 1968. Cheesemaking experiments concerning the effect of micrococci on the activity of propionic acid bacteria in Emmental cheese. Schweiz. Milchztg., 94, Wiss. Beil., 117: 977-988. (Dairy Sci. Abstr., 30: 388).

106. Ritter, P., H. Schwab, and H. Holzer. 1967. Testing the stimulatory or inhibitory effect of micrococci on propionic bacteria. Schweiz. Milchztg., 93, Wiss. Beil., 113: 929-930. (Dairy Sci. Abstr., 29: 461).

107. Ritter, W., K. W. Sähli, and P. Schilt. 1961. Residual sugar and pH values of fresh Emmental cheese. Schweiz. Milchztg., 87, Wiss. Beil., 77: 613-620. (Dairy Sci. Abstr., 26: 25).

108. Robertson, P. S. 1962. The estimation of carbon dioxide in Cheddar cheese. J. Dairy Res., 29: 321-323.

109. Rollman, N., and G. Sjöström. 1946. Undersökningar över några propionsyrabakteriestammars förhållande gentemot koksalt, salpeter och upphettning. Svenska Mejeritidn. 38: 199-201, 209-212.

110. Sadoff, H. L. 1966. Glucose dehydrogenases—soluble Bacillus cereus. Vol. IX, p. 103-107. In: Colowick and N. O. Kaplan (ed.) Methods in Enzymology, Academic Press, Inc., New York, N.Y.

111. Sähli, K. W., and W. Lehmann. 1959. Bacteriological and chemical studies on mature high-quality Emmental cheese. Schweiz. Milchztg., 85, Wiss. Beil., 64: 505-512. (Dairy Sci. Abstr., 21: 419).

112. Sanders, G. P., and R. R. Farrar. 1939. Some factors affecting the moisture content of Swiss cheese and their effects upon quality and yield. J. Dairy Sci., 22: 433-444.

113. Sanders, G. P., R. R. Farrar, R. E. Hardell, F. Feutz, and L. A. Burkey, 1940. The relationship of moisture in Swiss cheese to quality and yield. J. Dairy Sci., 23: 905-918.

114. Schultz, M. E., G. Sydow, and K. H. Leder. 1958. Die Anwendung der Röntgen-Diagnostik bei der Herstellung von Hart- und Schnittkäsen. Milchwiss., 13: 214-219.

115. Schwab, H., and V. Oehen. 1970. Oxidation of milk fat by copper and pigment formation by propionic acid bacteria. XVIII Int. Dairy Congr., IE: 375.

116. Sherman, J. M. 1921. The cause of eyes and characteristic flavor in Emmental or Swiss cheese. J. Bacteriol., 6: 379-391.

117. Sherman, J. M. 1923. The use of bacterial cultures for controlling the fermentation in Emmenthal cheese. Proc. World's Dairy Congr., 1: 287-290.

118. Sherman, J. M., and R. H. Shaw. 1921. Associative bacterial action in the propionic acid fermentation. J. Gen. Physiol., 3: 657-658.

119. Sherman, J. M., and R. H. Shaw. 1923. The propionic acid fermentation of lactose. J. Biol. Chem., 56: 695-700.

120. Stadhouders, J., and H. A. Veringa. 1967. Texture and flavour defects in cheese caused by bacteria from contaminated rennet. Neth. Milk Dairy J., 21: 292-297.

121. Steffen, C. 1968. Study of fermentation in model cheeses of different lactose content. Schweiz. Milchztg., 94, Wiss. Beil., 118: 991-993. (Dairy Sci. Abstr., 31: 27).

122. Stettler, H. 1963. Effect of centrifugation and filtration of cheese milk on eye formation in Emmental cheese. Schweiz. Milchztg., 89: 565-567. (Dairy Sci. Abstr., 26: 68).

123. Stine, J. B. 1950. Emmenthaler cheese. U. S. Patent 2,494,636. Offic. Gaz., 630: 710.

124. Storgårds, T., and M. Hietaranta. 1949. On the results of proteolysis in the ripening process of Emmenthaler cheese. XII Int. Dairy Congr., 2: 227-230.

125. Swartling, P. 1957. The packing of hard cheese in Cryovac. Svenska Mejeritidn., 49: 437-438. (Dairy Sci. Abstr., 20: 22).

126. Thomé, K. E., and B. Lindgren. 1948. Propionsyrabakteriernas värmetålighet under Lågpastöriseringsförhållanden. Medd. Statens mejeriforsok, 23: 1-25.

127. Tittsler, R. P. 1940. The influence of hydrogen-ion concentration upon the growth of Propionibacterium. J. Bacteriol., 39: 95-96.

128. Tittsler, R. P., and G. P. Sanders. 1953. Effects of pH on the growth of Propionibacterium shermanii and its relation to the quality of Swiss cheese. J. Dairy Sci., 36: 574-575.

129. U. S. Department of Agriculture. Crop Reporting Board, Statistical Reporting Service. 1970. Production of manufactures dairy products. Washington, D.C., U. S. Dept. Agr. Crop Reporting Board. July 19, 1970.

130. Uotila, H. 1965. Differences in the content of dry matter, fat, and NaCl of Finnish Emmental cheese in different parts of cheese. Meijerit. Aikakausk., 25: 97-105. (Dairy Sci. Abstr., 28: 1346).

131. Van Allemeersch, X., and R. Devogelaere. 1966. Radiographic studies on cheese. Meded. Rijksfak. Lanb. Wet. Gent., 31: 1231. (Dairy Sci. Abstr., 29: 4519).

132. Van Beynum, J., and J. W. Pette. 1943. Der Nachweis gasbildender Bakterien im Käse. Milchw. Zentralbl., 72: 81-82.

133. Van Niel, C. B. 1928. The propionic acid bacteria. J. W. Boissevain and Co., Haarlem, Netherlands. 187 p.

134. Vedamuthu, E. R., and G. W. Reinbold. 1967. The use of candle oats jar incubation for the enumeration, characterization and taxonomic study of propionibacteria. Milchwiss., 22: 428-431.

135. Vedamuthu, E. R., G. W. Reinbold, and E. G. Hammond. 1968. Inhibitory activity of acid and rennet whey on propionibacteria. J. Dairy Sci., 51: 503-510.

136. Virtanen, A. I. 1923. Über die propionsäuregarung. Soc. Sci. Fennica, Commentationes Phys.-Math., 1: 1-23.

137. Virtanen, A. I. 1939. pH in Emmental cheese during different stages of manufacture (in German, English summary). Meijerit. Aikakausk. 1/2: 21-26.

138. Von Freudenreich, E., and S. Orla-Jensen. 1907. Über die Emmentalerkäse stattfindende Propionsäuregarung. Zentralbl. Bakt. Parasitenk., 17: 529-546.

139. Watson, P. D. 1929. The relation of the hydrogen-ion concentration to the texture of Emmenthal or Swiss cheese. J. Dairy Sci., 12: 289-294.

140. Whittier, E. O., and J. M. Sherman. 1923. Propionic acid and ketones from whey. Ind. Eng. Chem., 15: 229-231.

141. Winkler, S. 1952. Über weitere antibiotische Wirkungen auf Propionsäurebakterien. Milchwiss. Ber., Wolfpassing, 4: 145-154.

142. Winkler, S. 1953. The inhibitory action of propionic acid bacteria. XIII Int. Dairy Congr., 3: 1164-1167.

143. Wold, F. 1971. Macromolecules: structure and function. Prentice-Hall, Inc., Englewood Cliffs, N.J. p. 88-98.

144. Wood, H. G. 1934. The physiology of the propionic acid bacteria. Ph.D. Thesis. Iowa State Univ. Libr., Ames, Iowa.

145. Wood, H. G., and C. H. Werkman. 1936. Mechanism of glucose dissimilation by the propionic acid bacteria. Biochem. J., 30: 618-623.

146. Ystgaard, O. M., A. H. Strand, E. Brandsaeter, and G. Syrrist. 1963. Ost av sveitserost-typen ystet av pasteurisert melk. (in Norwegian, English summary). Dairy Res. Inst., Agr. Coll., Norway, Rep. 108.

147. Ziegler, H. 1964. Ripening of Emmental cheese in plastic film. Nord. Mejeritidsskr., 30: 184-186.

148. Zollikofer, E., and J. Hoffman. 1962. Der Einfluss des Kupfers auf die Gärung, insbesondere auf den Säuerungsverlauf im Emmentalerkäse. XVI Int. Dairy Congr., 2: 765-769.

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APPENDIX

Table 15. Moisture and milkfat content of experimental Swiss cheeses[a]

Cheese lot designation	Strain no.	Moisture (%)	Milkfat (%)
A	129	40.33	28.5
B	59	41.35	26.5
C	59	40.81	25.5
D	129	41.93	24.5
E	59	42.97	23.5
F	129	43.25	25.0
G	59	41.35	26.5
H	129	40.82	27.0
J	35	43.43	26.0
K	1	42.31	25.5
L	129	41.08	28.5
M	83	42.03	27.0
N	129	40.96	27.0
O	59	41.32	27.0
P	83	44.22	26.0
Q	59	40.83	27.0
R	129	43.00	27.0
S	83	41.47	27.5
T	47	39.80	27.5
U	59	42.08	27.5
V	109	41.78	25.5
X	59	43.70	26.5
Y	129	43.51	26.5
Z	5	41.76	27.5

[a]Determinations were performed by the Iowa State University Food Product Analysis Laboratory. Determinations were made on samples taken before brining.

Table 16. Carbon dioxide production during curing by 50 kg lots of experimental Swiss cheese manufactured with various strains of Propionibacterium spp.

Days after manufacture	Cheese lot and Propionibacterium strain									
	A 129	B 59	C 59	D 129	E 59	F 129	G 59	H 129	J 35	K 1
	μ moles CO_2									
11	31	17	19	3	27	15	50	9	9	39
12	31	9	14	4	30	12	32	27	1	12
13	31	11	22	6	32	25	73	29	1	1
14	31	20	30	7	44	33	76	30	1	1
15	30	25	38	10	50	34	76	33	1	1
16	32	33	50	10	52	39	82	33	9	12
17	34	35	48	10	52	45	82	45	9	6
18	31	31	45	12	56	45	88	51	14	6
19	28	34	55	14	71	48	99	51	14	15
20	28	35	61	14	71	45	120	42	32	27
21	38	48	60	17	88	45	134	51	47	48
22	55	63	63	20	112	39	145	73	54	54
23	68	68	66	24	115	63	150	72	63	66
24	70	72	82	24	224	69	155	75	57	75
25	75	75	133	20	59	75	160	80	78	78
26	79	90	148	24	44	78	165	75	84	80

[a]Cheese removed from hot room.

Cheese lot and *Propionibacterium* strain													
L 129	M 83	O 59	P 83	Q 59	R 129	S 83	T 47	U 59	V 109	W 59	X 59	Y 129	Z 5
μ moles CO_2													
11	3	36	9	25	9	89	42	97	73	17	42	58	59
1	1	22	12	25	18	64	29	105	81	13	51	46	55
1	1	17	12	27	29	78	29	114	79	17	47	46	51
1	1	11	15	28	29	84	15	108	92	21	43	50	49
1	3	23	17	28	37	83	15	99	112	21	55	44	41
3	15	23	19	30	43	91	12	76	118	25	41	42	39
6	34	31	14	34	46	105	17	68	129	30	43	45	33
6	40	26	25	33	48	108	17	101	174	38	45	50	25
26	42	24	30	33	58	197	15	128	247	60	63	54	25
36	45	31	39	36	55	231	43	196	320	81	86	58	33
45	43	31	85	40	65	350	75	276	343	128	125	58	38
45	50	45	158	40	68	484	116	335[a]	498	265	207	58	50
44	54	43	181	42	75	562	174	156	353	363	372	59	55
44	37	46	57	48	90	573	341	53	98	265	216	63	67
52	31	50	30	70	40	606	624	34	62	119	55	67	75
52	26	52	29	92	38	500	669	36	62	73	38	63	80

Table 16. (Continued)

Days after manufacture	Cheese lot and *Propionibacterium* strain									
	A 129	B 59	C 59	D 129	E 59	F 129	G 59	H 129	J 35	K 1
	μ moles CO_2									
27	84	123	160	36	32	95	170	73	112	80
28	95	185	180	50	15	115	171	78	115	89
29	110	37	193	63	15	120	175	80	142	75
30	50	23	208	90	18	125	190	96	115	102
31	17	34	108	101	20	39	222	98	86	153
32	10	30	66	101	26	21	263	99	57	144
33	7	32	63	98	28	12	146	102	23	96
34	10	28	60	117	30	15	59	117	14	60
35	7	30	53	60	30	17	20	126	14	15
36	10	28	50	40	32	19	14	117	11	20
37	3	28	47	34	30	18	22	125	12	20
38	10	23	35	20	30	18	17	135	13	19
39	10	21	32	20	32	18	12	60	18	19
40	10	21	32	17	31	18	13	15	18	18

Cheese lot and *Propionibacterium* strain													
L 129	M 83	O 59	P 83	Q 59	R 129	S 83	T 47	U 59	V 109	W 59	X 59	Y 129	Z 5
μ moles CO_2													
54	53	53	29	102	42	150	380	43	69	64	35	83	86
54	48	58	12	151	42	100	184	45	57	64	51	89	90
54	61	64	25	203	37	60	122	46	57	60	47	98	110
79	53	60	11	245	26	53	91	45	55	51	45	105	120
88	85	78	21	145	29	50	81	39	58	56	49	122	129
114	90	86	21	35	24	53	97	43	62	47	45	140	145
128	92	104	17	35	30	55	96	42	66	56	45	148	58
138	141	130	14	35	26	62	99	42	61	56	41	83	19
164	142	74	19	38	26	48	99	40	61	56	37	77	4
168	150	61	20	35	20	54	93	60	57	53	37	12	6
166	170	46	17	32	18	55	96	43	57	51	41	12	6
155	190	46	22	36	19	55	96	45	56	54	49	12	6
36	240	41	20	40	28	64	94	47	57	54	49	8	14
45	139	44	20	45	21	62	93	48	53	56	45	14	12

Table 16. (Continued)

Days after manufacture	Cheese lot and Propionibacterium strain									
	A 129	B 59	C 59	D 129	E 59	F 12[illegible]	G 59	H 129	J 35	K 1
	μ moles CO_2									
45	7	25	30	13	30	.8	15	14	24	19
50	11	16	35	10	28	20	18	12	20	30
55	10	15	35	30	24	10	15	10	20	25
60	10	19	50	32	22	17	13	7	15	25
65	10	20	55	30	21	14	15	8	10	25
70	10	18	50	26	20	13	18	10	9	15
75	10	20	40	23	22	15	17	9		
80	8	20	35	17	24	11	15	11		
85					20	16	14	8		
90					22	14	17	7		

Cheese lot and *Propionibacterium* strain													
L 129	M 83	O 59	P 83	Q 59	R 129	S 83	T 47	U 59	V 109	W 59	X 59	Y 129	Z 5
μ moles CO_2													
45	35	28	25	41	18	68	84	49	49	51	50	14	13
38	27	27	25	44	27	58	85	51	48	47	46	15	12
25	25	29	22	40	29	55	87	43	45	42	50	14	13
15	29	31	20	42	29	60	95	46	44	47	51	12	11
13	28	29	23	40	31	65	93	51	48	45	46	12	13
10	28	31	25	46	26	63	85	54	51	47	49	11	16
		29	22				87	48	50	45	50	12	16
		33	24				80	45	50	51	53	12	15
		35	20				86	46	51	47	53	10	13
		39	22				88	50	48	47	52	10	15

Table 17. Type and properties of packaging films used in the investigation

Film designation	Type of film	Supplier	Oxygen permeability[a]
I	Cryovac SW Clear 60 Type WS201	W. R. Grace Co., Simpsonville, S. C.	5.0
II	Double Cryovac SW Clear Type WS201 and paraffin	W. R. Grace Co., Simpsonville, S. C.	1.0
III	Nealam 60954[b]	Milprint, Inc., Milwaukee, Wis.	5.5
IV	Saranex 29 duplex	Kraftco Corp., Glenview, Ill.	0.5
V	Saranex 29 simplex and Polyethylene	Kraftco Corp., Glenview, Ill.	5.0
VI	Polyethylene duplex	Kraftco Corp., Glenview, Ill.	100.0
VII	Polyester/Saran/ Polyethylene	Kraftco Corp., Glenview, Ill.	1.0

[a]Approximate transmission rates of cc of oxygen/100 sq in/24 hr at 1 atm at 25 C.

[b]Nealam 60954 has a carbon dioxide transmission rate of 26.0 cc/100 sq in/24 hr at 1 atm at 25 C.

Table 18. Example of make record for Iowa Swiss-type cheese

Vat: X Made by D. H. and R. J. Date: February 10, 1971

Milk: Lbs 4900 Percent fat: 2.75

HEAT TREATMENT: Date 2/10/71 Time: 16 sec Temp: 161 F

STARTERS

	Strain	Date Set	% Acidity	Amount	Media
Lactic	253	2/9	0.92	4 lb	Milk
Coccus	A	2/9	0.85	12 lb	Milk
Rod	B	2/9		100 ml	Milk
Prop	P-59	2/8		400 ml	Broth

Rennet: 370 ml

MAKE PROCEDURE

Operation	Time	Temp. (F)	Acidity
Received in vat	6:30	40	0.16
Starter added	8:25	88	0.17
Rennet added	8:55	90	
Cut	9:35	90	0.09
Whey drawn — begin	9:40		
end	9:45		
Cook — begin	10:05	90	
(water temp 145) end	10:20	106	0.65
Vat press — begin	11:35	105	0.75
end	12:35		
Hoop press — begin	12:40		0.11
5 hr	5:40		0.34

Date into brine tank 2/11/71 Date out of brine tank 2/13/71

Date into warm room 2/20/71 Date out of warm room 3/6/71

Yield: 21 - 20 lb blocks