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# Development of a Whey-Based Lactic Culture Medium Capable of Bacteriophage Inhibition

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# DEVELOPMENT OF A WHEY-BASED LACTIC CULTURE MEDIUM CAPABLE OF BACTERIOPHAGE INHIBITION

by

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Chao T. Cheng

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Development of a Whey-based Lactic Culture Medium

Capable of Bacteriophage Inhibition

by

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A whey product has been formulated for use as a lactic starter medium. Phosphate-treated whey medium (PWM) has been shown to support growth of lactic cultures and prevent phage proliferation. Comparisons were made of a commercial phage inhibitory medium (PIM), reconstituted non-fat dry milk (NDM) and PWM. PWM inhibited all phages tested and stimulated starter growth. PWM was not as stimulating as PIM but was better than NDM. Good Cheddar cheese has been made using PWM. PWM is more economical than PIM but NDM is the most economical one if cheese yield is considered.

(49 pages)

# INTRODUCTION

It has been said with considerable truth the lactic starter culture is the heart of cheese making. No matter how perfect all other aspects may be, a bad starter is almost certain to give a poor quality cheese. A good starter may enable other defects, caused by a contaminated milk, to be overcome. Lactic starters which do not produce acid uniformly every day present an important problem to the dairy industry.

The media commonly used to prepare starter in cheese factories today include:

(a) Carefully selected whole or skimmilk from the factory.

(b) Reconstituted non-fat dry milk (NDM), pretested for ability to support lactic culture growth.

(c) Phosphate treated NDM media.

(d) Whey.

The latter is used only to a limited degree and primarily in Italian and Swiss cheese factories (17, 69).

The predominant media which have been used in dairy industry include reconstituted NDM and phosphate treated NDM. These have the following advantages:

(a) They are pretested and therefore free from inhibitory substances.

(b) They provide curd formation upon acidification which results in a visual indicator of culture quality.

(c) They closely resemble cheese milk and can increase product yield.

(d) The phosphate treated NDM inhibits bacteriophage (phage) proliferation.

The use of whey has been de-emphasized or completely forgotten for these reasons:

(a) Lack of curd formation thus requiring that other tests be employed to measure the culture quality.

(b) Presence of culture by-products and other chemical inhibitors.

(c) Lower activity of culture in this substrate unless modified.

(d) Possible presence of phage and undesirable contaminants.

(e) Lack of control by those preparing cultures.

However, whey is produced at the rate of more than 19 billion pounds annually (54) of which approximately 75-80 percent is disposed of as waste (Ernstrom).[1] The discarded whey represents a large reservoir of protein, sugar and minerals. Conversion of this waste into utilizable products would help relieve many plants and municipalities of a serious waste disposal problem (61, 62). If whey could be used as a bulk culture medium it would eliminate the need for purchase of more expensive culture nutrients and provide a practical outlet for whey solids.

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[1] C. A. Ernstrom, Head of the Department of Food Science and Industries, Utah State University, Logan, Utah. Personal communication, Fall, 1969.

The purpose of this research has been to develop an economical whey-based medium which would encourage the growth of lactic cultures and prevent phage proliferation.

# REVIEW OF LITERATURE

## Milk for starter media

Sellars (50) estimated that over 215 million pounds of milk were used in the U.S.A. for the production of lactic starter in 1965. With the increase in cheese production this figure was probably 223 million pounds in 1968.

Whole and skimmilk are standard media for starter propagation. These media supply essential vitamins and nitrogenous substances for many lactic acid bacteria (48). Disadvantages of using these media arise when they:

(a) Vary in composition (i.e., solids not-fat, peptone and peptide fractions) (2, 50, 52).

(b) Allow phage attack (3, 4, 12, 13).

(c) Contain antibiotics or other inhibitors (5, 20, 48).

Spray dried NDM is also reconstituted for the preparation of starter. The advantages of this product include economy, uniformity of composition and near-absence of inhibitory substances and undesirable organisms (17). The rate of acid formation and flavor development is comparable between normal skim milk and properly reconstituted NDM (50). But some reports indicate that reconstituted NDM gives higher and more consistent culture activity than pasteurized skim or whole milk (48). This medium also supports phage attack.

The importance of phages to the dairy industry

Phage infection of lactic starters has been the most important cause of insufficient acid production during controlled dairy fermentations (29, 60). In addition to disruption of carefully timed schedules in the cheese plants which cause "slow" or "dead vats," production of insufficient lactic acid may lead to undesirable fermentations causing abnormal fruity, rancid and putrid flavors and gas formation. A slow vat may also allow staphylococcal growth and toxin formation (18, 57).

Whey is the most important source of phages. Whey contact with equipment and whey mists make phage particles ubiquitous. It is inevitable that phage contaminates cheese operations (5, 59). Phage can survive for over ten years in soil and for more than two years on cheese shelves (17). The contamination of starters by wind-borne droplets is, therefore, possible not only from whey in the factory but from air currents near the factory.

Electron micrographs of a phage active against Streptococcus lactis have been prepared (44). The phage particles are sperm-shaped, 220-230 mμ long, with a head diameter of 70 mμ and a tail that is 20-30 mμ wide and 150-160 mμ long (8, 12, 18, 44). Nine strains of micrographed phage particles were so nearly alike, they could not be differentiated.

Micrographs of phages associated with cells indicated orientation with the tail toward the bacterial cell. A possible sequence for phage lysis of S. lactic cells was described by Parmelee et al. (44).

Phage races usually show a wider diversity of reaction of temperature conditions than do the homologous organisms. The optimum growth temperature for S. cremoris is near 30 C. These organisms are usually inhibited at 37 C. Some phage strains developed as well at 22 C as at 30 C. Most phage strains developed more rapidly at 30 C than 22 C. Some developed more readily at 37 C than at lower temperatures and others were completely inhibited at 37 C (31).

Physiological and chemical properties of phage

Lysis of bacteria can be controlled by medium acidity. Lysis was most rapid at pH 6.5 to 7.0, somewhat slower at pH 6.0 and 8.0 and almost completely inhibited below pH 5.0 (18, 43).

Phages are harder to inactivate with chemical and physical agents than their host cells. They can survive ordinary pasteurization but are destroyed during 70 to 75 C for 30 min at pH 6.0 (59). It is thus possible to destroy phage in whey by heat, but phage contaminated surfaces may form on vats, floors and walls that are subject to splashing (32). (Ethylene) glycol, aerosols of calcium hypochlorite, other hypochlorite germicides, and quaternary ammonium compounds have been used to destroy phage (7, 18, 32). Hypochlorite was the most effective on plant equipment, utensils, and building surfaces. One gram of available chlorine per 1,000 $ft^3$ of air space has been recommended for the inactivation of air-borne phage (18).

Ultraviolet irradiation of S. lactis and S. cremoris phages indicated that destruction may occur but irradiation is impractical in dairy plants (24).

Phages are very highly strain specific but strains of lactic organisms vary in their resistance. The nascent phenomenon apparently occurs with lactic cultures (3, 12). When a resistant strain and a sensitive strain become infected with a specific phage, both strains may be inhibited or lysed.

The action of phages on lactic streptococci may vary widely and is not in all cases an all or nothing phenomenon. There are five possibilities of phage activity spectrum-ranging from no action through the nascent effect, host-controlled variation, full action and host range mutation (12).

Detection of phage

It is wise to detect phage existence periodically in the plant before it develops into a severe infestation. Simple tests may be employed in the plant to provide presumptive evidence of phage presence.

A few drops of fresh starter may be added to 10 ml of sterile skim milk in a tube. If the milk fails to coagulate in 24 hr, the possibility of phage in the starter exists. If a second tube of milk is inoculated in the same manner and incubated at 86 to 98.6 F, microscopic examinations of the contents can be made at intervals over a period of about 8 hr. If the organisms begin to multiply and then lysis is noted, this is good evidence that phage has attacked and destroyed the bacteria. If phage or inhibitory substance is suspected in cheese manufacture, Elliker (18) described an easy method to detect or distinguish them. Triplicate tubes or small flasks containing sterile skim milk may be used. The first and second containers are inoculated with 2 or 3 drops of whey from a suspected vat.

The third one serves as a control. After the second container is heated to boiling for five minutes to inactivate phage and cooled, all three may be inoculated with 0.5 percent of fresh starter and incubated at 86 F for 6 hr. The titratable acidity is then determined. If starter in both the first and second containers are inhibited, it is probably a heat stable antibiotic such as penicillin, nisin or diplococcin. If the only starter inhibited is in first container, it is probably phage or a heat labile antibiotic. Other assays are possible (27) but are not practical for most cheese plant operations.

Phages and other inhibitors

The relative importance of phage, inhibitory substances and sanitizing agents as causes of cheese culture failure has been studied by Moseley and Winslow (42). They examined samples of milk, lactic cultures and whey obtained from 91 cheese factories in 20 states. The samples were obtained at the time of slow acid development during cheese manufacture. Their study involved 101 cases of culture failure. They also analyzed samples taken from 23 vats in 20 cheese factories that were not experiencing difficulty with slow acid development. Phages were found in 93 percent of the culture failures and 74 percent of the non-failures. Inhibitory substances in cheese milk caused 27 percent of the failures and 10 percent of the non-failures (both phage and inhibitory substances caused some failures). Sanitizing agents were relatively unimportant.

General approaches to eliminate phage infection

Many recommendations have been made to limit phage entrance into cultures and to decrease its activity (6, 12, 18). These include culture selection, effective sanitation program, improved culture propagation facilities, culture rotation, culture combination and preparation and propagation of cultures in a relatively phage-free area. Where facilities are available, tests may be run on whey or other products to ascertain if phage is accumulating against a certain strain. These methods have been helpful in decreasing culture failures but have not entirely eliminated the phage problem. Recent researchers have developed a medium that supports growth of lactic cultures, but does not permit phage development. "Marstar"[1] and "Culture Mate"[2] are commercial examples of these products (29, 50). It has been estimated that over 3 million dollars worth of "Marstar" was marketed in 1966 (Richardson).[3]

Calcium ion requirement for phage proliferation

Little is known of the effect of media constituents upon phage proliferation. The requirements may be different than those of the host cells. Cherry and Watson (8, 9) in 1949 reported that tryptone, calcium and many other electrolytes promoted lysis of S. lactis cells by phage. Collins et al. (14) found that calcium, amino acids

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[1]Trademark of Marschall Dairy Laboratory, Inc., Madison, Wisconsin.

[2]Trademark of Galloway-West Co., Fond du Lac, Wisconsin.

[3]G. H. Richardson, Professor of Food Science and Industries, Utah State University, Logan, Utah. Personal communication, 1966.

and vitamins were necessary for the multiplication of many phages active against strains of lactic streptococci. They suggested a medium for carrying lactic streptococci using chemically defined nutrients free of calcium. Complex nutrients low in calcium content and supplemented with phosphate or other calcium-binding ion were also suggested (14). Shew reported in 1949 that lactic streptococci phage required calcium for maximum development (51). Potter and Nelson (45, 46) in 1952 prepared a calcium deficient, partially chemically defined medium in which proliferation of six phage strains was prevented unless soluble calcium was added.

The function of calcium ion in phage multiplication is not clear. Adams (1) in 1959 reported that calcium seemed to be required for some stage of the infectious process subsequent to adsorption of virus to the host cell. Potter and Nelson (45) found that the stimulatory effect of calcium could not be explained by its effect upon adsorption. Under the experimental conditions employed, 85, 89, and 75 percent of the particles of three phage strains studied were absorbed during a 10 min period in the absence of calcium but no phage proliferation occurred. Slightly greater adsorption occurred when calcium ion was present, but the effect on proliferation was out of proportion to the effect upon adsorption. Other investigators have ascribed the effects of calcium removal to a reduced activity of certain enzymes that may be necessary for phage multiplication (14).

Calcium free medium

The first commerical phage resistant medium (PRM) was developed by Reiter in England (6, 12). Calcium was removed by ion exchange and

replaced by a nonessential ion. Unfortunately, essential elements were apparently also removed because many cultures did not grow well in PRM (6, 12).

Phosphate-treated medium

U.S.D.A. workers (25, 26) reported the effects of various phosphate salts on binding calcium and preventing phage proliferation in milk cultures. The type and concentration of phosphate, the pH, and heat-treatment markedly affected phage inhibition and the free calcium content of milk. Orthophosphates produced the greatest degree of phage inhibition. Most of the phage types tested were suppressed by two percent orthophosphate salts but the most resistant types required three percent. Heating the milk after phosphate addition was essential to bind most of the free calcium. The free calcium content of the treated milk ranged from 10 to 30 ppm. Inhibition became more pronounced as the phosphated milk was adjusted from pH 6.4 to 7.0.

The best combination for phage inhibition, minimum milk precipitation and economy was obtained when milk was heated with 1.7 percent orthophosphate salt at pH 6.6; followed by the addition of 0.3 percent pyrophosphate. Thirteen different lactic strains, which were grossly contaminated with their respective phage, were freed of phage within three to four subcultures in phosphated milk. In most instances, the activity of the cultures in phosphated milk was as great as or greater than the controls.

Kadis and Babel (34) added mixtures of phosphates to skim milk or reconstituted skim milk. The use of 36 g $KH_2PO_4$ plus 24 g $Na_2HPO_4$ per

100 ml milk yielded a medium which prevented phage development in three out of nine starter cultures and reduced the rate of phage proliferation in four of the remaining six cultures. Coagulation of milk after sterilization was encountered with the following mixtures:

(a) 30 g $KH_2PO_4$ plus 30 g $Na_2HPO_4$ in 100 ml.

(b) 20 g $KH_2PO_4$ plus 40 g $Na_2HPO_4$ in 100 ml.

Addition to 100 ml milk of (a) 40 g $K_2HPO_4$ plus 20 g $NaH_2PO_4$ or (b) 48 g $K_2HPO_4$ plus 12 g $Na_2HPO_4$ proved inhibitory to many of the lactic starter cultures tested. Thus simple addition of phosphate to milk was inadequate to produce a suitable medium that would inhibit phage and still permit satisfactory bacterial growth.

Effect of stimulatory substances on starter culture growth in milk

Dahiya and Speck (16) have found that milk is deficient in nucleic acid derivatives for optimum growth of lactic streptococci. Growth can be accelerated through supplementation of milk by enzymatic hydrolysates or proteins (2, 22, 52) and by crude extracts of various plant and animal tissues (e.g., liver fractions, yeast extract, peptone, pancreas extracts and corn steep liquor, etc) (22, 35, 53).

Speck et al. (53) further reported that liver fractions, yeast extract and pancreas extract all contained multiple stimulants for most cultures of lactic streptococci. Four factors were found in all three extracts, a fifth factor was present in liver and yeast extracts but not in pancreas extract. Two of the factors in pancreas extract have been identified as peptides, presumably the other factors

are also peptides. The latter react with ninhydrin and activity is lost upon acid hydroplysis. Later, Koburger et al. (36) identified adenine, hypoxanthine and inosine as active components in pancreas extract that contributed to growth-promoting properties.

Phosphated medium with stimulatory agents

Simple phosphated medium as developed by Hargrove et al. (26) was not a satisfactory medium for the industry. Stimulatory substances were needed for optimum growth of starter cultures (16, 22) and phosphates caused milk protein precipitation during preparation (34). Several mono- and dibasic orthophosphate salts, dry-blended with yeast extract and NDM, were evaluated by Zottola and Marth (63). As a result of their study, three combinations were found that inhibited most of the phages tested:

(a) A 3:2 mixture of NDM and electro-dialyzed whey (EDW) with a two percent orthophosphate concentration (reconstituted basis) consisting of a 1:1 ratio of $Na_2HPO_4$ and $KH_2PO_4$.

(b) A 4:1 mixture of NDM and EDW with a two percent orthophosphate concentration (reconstituted basis) consisting of a 1:1 ratio of $Na_2HPO_4$ and $NH_4H_2PO_4$.

(c) A 4:1 mixture of NDM and EDW with a two percent orthophosphate concentration (reconstituted basis) consisting of a 1:1 ratio of $NH_4H_2PO_4$ and $(NH_4)_2HPO_4$.

The dairy ingredients were reconstituted to 9-10 percent in each medium. All of the mixtures contained 0.32 percent yeast extract (reconstituted basis).

The first two formulae inhibited five of six phages examined, whereas, the third effectively inhibited all six. None of the three mixtures was inhibitory to the host lactic streptococci.

PIM

Recently, a new phage inhibitory medium (PIM) has become available This medium, called "Marstar" or "Culture Mate" (see page 9, footnotes 1 and 2 of this thesis), is a scientifically blended combination of a high grade NDM, de-mineralized whey powder, certain phosphate salts, extracts and growth stimulants (dried pancreas extract) (38).

The ability of PIM to minimize phage development in single or mixed strains lactic starter cultures and to dilute out phage particles from infected cultures has been confirmed (29). Single strains of S. lactis, S. cremoris and S. diacetylactis grew well in PIM though there was variation between strains.

Whey for preparing starters

Whey contains about 6.5 to 7.0 percent solids (20, 33) following removal of the casein, fat and some lactose and minerals from milk. This substrate has been used traditionally for preparing starters in Italian and Swiss cheese plants. Excellent quality cheese can be produced if whey starters are properly prepared and maintained in good consition (49). Reports of autoinhibition of lactic streptococci (23) and concern over strain domination and antibiotic production (11) have caused concern over the use of spent whey

for culture propagation. However recent workers (15) have successfully used it for preparing cell concentrates.

EDW accounted for 40 percent of the milk solids in the media formulated by Zottola and Marth. All three combinations contained EDW (63). PIM apparently also contains a certain amount of EDW (38).

# EXPERIMENTAL METHODS

## Lactic streptococci cultures and homologous phage filtrates

Several strains of lactic streptococci were obtained along with their homogous phage races from D. P. L. Culture Service, 1750 Folsom St., San Francisco 94103. These were strains SK-11, KH, R-1, $C_2$, and $C_6$. The phage for strain $C_6$ was found to be very weak, therefore, only four strains were studied extensively.

The lactic streptococci were maintained in 10 ml of 10 percent sterilized (121 C for 15 min) reconstituted NDM. They were transferred weekly using one percent inoculum and incubated for 16 hr at 21 to 22 C.

Phage lysates were prepared as follows: 50 ml of sterilized and cooled Elliker lactic broth (19) supplemented with one percent sterile 1 M $CaCl_2$ (1, 9) were inoculated with 1 percent culture and incubated at 30 C for 2 to 3 hr until the broth was slightly turbid indicating an early log phase of culture development (27). The cultured broth was infected by one percent of homologous phage filtrate. The phage infected broth was incubated overnight at room temperature (25 C). In most cases, the lysates were clear when compared to a non-infected control culture. The phage hysates were centrifuged for 15 min and filter-sterilized sequentially through two membrance filters (28, 63) with average pore diameters of 45 and 22 mμ (Millipore Filter Corp., Bedford, Mass.). This treatment removed bacterial cells and other particles. The resulting phage filtrates were maintained at 2 to 4 C until used (10, 63).

The phage titer was estimated by a dilution end point method in sterile litmus milk (1, 21).

Preparation of phosphate-treated whey medium and other media used for comparison

Fresh whey was obtained from commerical Cheddar cheese factories. Phosphate-treated whey medium (PWM) was prepared by adding and dissolving the following chemical agents as reported by Zottola and Marth (63): 0.32 percent yeast extract, (Adramine 99 or Yes,)[3] one percent $NH_4H_2PO_4$, one percent $Na_2HPO_4$ (all on a reconstituted basis). The medium was transferred using a 10 ml pipette into sterilized test tubes. The contents and test tubes were heat-treated for 45 mins in an 88 to 90 C hot water bath (63). A thermometer was inserted into a dummy tube to indicate the applied temperature. After heat-treatment, the tubes were immediately cooled to 29 or 21 C and inoculated (26, 63).

"Marstar," a popular commerical phage inhibitory medium (PIM), was prepared according to the manufacturer's directions (38) and in the same manner as PWM. Reconstituted 10 percent NDM and unmodified whey were sterilized in like manner.

Lactic acid production test

The lactic culture activity test was conducted as follows: The four media (whey, PWM, PIM, and NDM) were prepared and evaluated simultaneously.

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[3]Trade name of Yeast Products Inc., Paterson, New Jersey.

Each medium was prepared in a set of nine tubes containing 10 ml, one was used as an uninoculated control and the others were divided into four pairs. The first of each pair was inoculated with lactic streptococci only and the second was inoculated with culture plus homologous phage filtrate. A one percent inoculum was used for both culture and phage filtrate. All tubes were incubated at 29 C for 16 hr (63). After incubation the titratable acidity (TA) of all sets and controls were determined, using the Milk Industry Foundation procedure (41).

Starter activity test

Ability to produce acid during 16 hr incubation did not indicate potential value in the cheese vat. Therefore the activity of starter cultures under conditions stimulating those in the cheese vat was determined using the method of Horrall and Elliker (30).

High-grade spray-dried NDM was reconstituted at the rate of 10 g per 90 ml of distilled water. It was sterilized in flasks at 121 C, for 10 min. Ten milliliters were pipetted into sterile screwcapped test tubes and adjusted to 37.7 C in a water bath. Each tube was incoulated with 0.3 ml of the starter culture to be tested and incubated at 37.7 C for 3.5 hr. At the end of incubation, the entire contents of the tube, together with 5 ml of distilled water used to rinse the tube, were titrated with 0.1 N NaOH to a faint pink color, using phenolphthalein indicator. The results were recorded as percent lactic acid.

Bacteria enumeration

The number of viable bacteria present in the starter were estimated by determining the Standard Plate Count (55). Elliker lactic medium was used to enhance growth (19).

Method of Cheddar cheese manufacture

Cheddar cheese was manufactured in the USU Dairy Products Lab. using conventional procedures suggested by Price and Calbert (47) except that two percent starter was added and the ripening period was eliminated.

# RESULTS AND DISCUSSION

## Determination of phage titer

Phage was titered using the dilution end point method in sterile litmus milk. The titer of phage present in the original filtrate was calculated after 16 hr incubation period at 30 C. The growth condition was also observed after 24 hr incubation. The highest dilution inhibiting the litmus milk culture (demonstrated by lack of acid coagulation and reduction) provided an estimate of phage population. Data in Table 1 summarizes phage titers after 16 and 24 hr incubation at 30 C. The titer was lower after 24 hr indicating that resistant cells were active. Phage $c_6$ was deleted from subsequent study because of inactivity.

Table 1. Phage titers estimated with the dilution end point method in sterile litmus milk. (Average of three replicates.)

| Phage races | Titer/ml | |
|---|---|---|
| | I[a] | II[b] |
| sk-11 | $10^7$ | $10^5$ |
| kh | $10^7$ | $10^6$ |
| r-1 | $10^7$ | $10^6$ |
| $c_2$ | $10^{10}$ | $10^8$ |
| $c_6$ | $10^2$ | 0 |

[a]Calculated after 16 hr incubation at 30 C.
[b]Calculated after 24 hr incubation at 30 C.

Comparison of media for starter growth and phage inhibition

The significance of phosphate treatment and yeast addition to whey was determined. Four media were compared for their effectiveness in preventing phage proliferation and also their effect on the growth stimulation of lactic streptococci. The acidity (expressed as percent lactic acid) that developed after a 16 hr incubation period at 29 C was used as the criterion of culture activity and phage proliferation (63). These comparisons are presented in Table 2 and Table 3. It is assumed that the greater the developed acidity, the more active the culture and the less active the phage. The developed acidity was obtained by substracting the TA of the uninoculated control from the TA of the inoculated or phage-infected samples. The incubation temperature used in the test was chosen to obtain maximum phage activity and the incubation time used was required for maximum acid production in phosphate-treated milks (63).

PWM was definitely more effective in preventing phage proliferation than whey and had more stimulatory effect upon lactic culture activity. Thus phosphate treatment and yeast addition were beneficial. Phage activity was inhibited in both PWM and PIM. Conversely, in the conventional media, bacterial acid production was seriously retarded with all bacterial strains.

These results support the findings of Zottola and Marth (63) and Hargrove et al. (26) who reported that phosphate treatment would inhibit phage proliferation. I confirmed that PWM, PIM and reconstituted NDM

Table 2. Comparison of starter activity and phage inhibition in various media including PIM (16 hr incubation at 29 C)

| | | Developed acidity in percent | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I[a] | | II[b] | | III[c] | | IV[d] | |
| Culture | Trial | Phage free | Phage added | Phage free | Phage added | Phage free | Phage added | Phage free | Phage added |
| SK-11 | 1 | .20 | .07 | .57 | .54 | .63 | .65 | .71 | .04 |
| | 2 | .24 | .06 | .60 | .65 | .64 | .69 | .74 | .16 |
| | 3 | .19 | .08 | .66 | .65 | .67 | .69 | .69 | .14 |
| | 4 | .16 | .04 | .58 | .58 | .60 | .56 | .66 | .11 |
| | Ave. | .198 | .063 | .603 | .605 | .635 | .648 | .700 | .113 |
| KH | 1 | .25 | .16 | .68 | .69 | .78 | .79 | .66 | .29 |
| | 2 | .25 | .12 | .72 | .65 | .83 | .83 | .71 | .22 |
| | 3 | .24 | .14 | .74 | .71 | .80 | .80 | .62 | .27 |
| | 4 | .20 | .08 | .68 | .66 | .85 | .79 | .64 | .11 |
| | Ave. | .235 | .125 | .705 | .678 | .815 | .803 | .658 | .223 |
| R-1 | 1 | .25 | .18 | .87 | .87 | .95 | .98 | .75 | .04 |
| | 2 | .24 | .16 | .74 | .74 | .90 | .85 | .73 | .13 |
| | 3 | .24 | .11 | .81 | .81 | .94 | .95 | .78 | .24 |
| | 4 | .22 | .07 | .75 | .76 | .89 | .67 | .67 | .16 |
| | Ave. | .238 | .130 | .793 | .795 | .920 | .863 | .733 | .143 |
| $C_2$ | 1 | .28 | .03 | .83 | .83 | .98 | .95 | .75 | .02 |
| | 2 | .23 | .01 | .80 | .80 | .93 | .90 | .73 | .10 |
| | 3 | .26 | .08 | .84 | .82 | .94 | .98 | .78 | .06 |
| | 4 | .24 | .05 | .76 | .75 | 1.00 | .02 | 1.72 | .02 |
| | Ave. | .253 | .043 | .808 | .800 | .963 | .963 | .745 | .050 |

Table 3. Comparison of starter activity and phage inhibition in various media PIM-1 (16 hr incubation at 29 C)

| | | Developed acidity in percent | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I[a] | | II[b] | | III[c] | | IV[d] | |
| Culture | Trial | Phage free | Phage added | Phage free | Phage added | Phage free | Phage added | Phage free | Phage added |
| SK-11 | 1 | .18 | .04 | .61 | .59 | .62 | .63 | .70 | .12 |
| | 2 | .21 | .08 | .63 | .59 | .65 | .63 | .71 | .14 |
| | 3 | .20 | .08 | .60 | .58 | .62 | .60 | .68 | .14 |
| | Ave. | .197 | .067 | .613 | .587 | .630 | .620 | .697 | .133 |
| KH | 1 | .17 | .08 | .71 | .70 | .68 | .68 | .65 | .08 |
| | 2 | .23 | .03 | .70 | .68 | .71 | .70 | .67 | .15 |
| | 3 | .18 | .06 | .68 | .66 | .72 | .70 | .63 | .15 |
| | Ave. | .193 | .057 | .697 | .680 | .703 | .693 | .650 | .127 |
| R-1 | 1 | .20 | .05 | .74 | .71 | .76 | .73 | .62 | .10 |
| | 2 | .20 | .07 | .76 | .75 | .79 | .76 | .66 | .14 |
| | 3 | .21 | .05 | .76 | .74 | .78 | .74 | .71 | .08 |
| | Ave. | .203 | .057 | .753 | .733 | .777 | .743 | .663 | .107 |
| $C_2$ | 1 | .20 | .06 | .76 | .73 | .74 | .76 | .70 | .08 |
| | 2 | .24 | .06 | .82 | .78 | .79 | .79 | .70 | .08 |
| | 3 | .23 | .04 | .82 | .82 | .82 | .81 | .74 | .08 |
| | Ave. | .223 | .053 | .800 | .777 | .783 | .787 | .713 | .080 |

[a]Whey
[b]PWM
[c]PIM
[d]NDM
[e]PIM-1

were suitable as culture media. The latter was the best medium for growth of phage free SK-11. This was probably due to some inhibitory substances or lack of a growth factor in the other two media for this particular strain. Three of the cultures grew better in PWM and PIM than in NDM. Yeast and pancreas extracts provide additional nutrients and stimulants for lactic culture growth. In all cases, PIM was superior to PWM. Two batches of "Marstar" PIM were evaluated. One lot (unavailable No.) was received in May, 1967 (PIM-1). The other lot (No. 0173029) was received in October, 1969 (PIM). Different degrees of stimulation were observed for the two lots (Tables 2 and 3). PWM was as stimulatory as PIM-1. PIM was found to be more stimulatory than either PIM-1 or PWM.

The manufacturer was contacted. He explained that a new formulation was involved in recent batches of "Marstar." He could not reveal the nature of the change but indicated that it produced a higher initial TA and stimulated culture growth and acid production (Christensen, personal communication).[1]

The differences between PWM and PIM might be due to physical state (39, 40) and nutrient contents of the media. More work is needed to establish the reasons for these observations.

Tests using the NDM medium indicated no appreciable acid development during the incubation period after inoculation with culture and homologous phage. Therefore, the procedure for evaluating culture and phage activity was believed to be satisfactory.

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[1] D. R. Christensen, Technical Director, Marschall Dairy Laboratory, Inc., Madison, Wisconsin. Personal communication, 1969.

Effect of various media on lactic starters for Cheddar and Cottage cheese making

A lactic culture must actively produce acid at a controllable rate following inoculation into the cheese milk. The possible use of PWM bulk culture in commercial cheese factories was first screened by the method of Horrall and Elliker (30).

Lactic starters were prepared in PWM, PIM and reconstituted NDM, by heating at 88 to 90 C min, cooling, inoculating and incubating at 21 C for 16 hr to 18 hr. They were then inoculated (0.3 ml/10 ml of 10 percent NDM) into 37.7 C substrate, incubated 3.5 hr and titrated along with 5 ml rinse water to the phenolphthalien end point using 0.1 N NaOH.

All lactic starters produced a TA over 0.3 percent and therefore were interpreted as satisfactory for use in Cheddar and Cottage cheese (Table 4). Three out of five strains were very active in both PIM and PWM and produced TA values over 0.4 percent. $C_2$ produced a high of 0.62 percent. These high values confirm the suggestion that smaller bulk inocula are required with PIM type media. Only one strain was found inactive in reconstituted NDM medium. However, satisfactory cheese timing would be possible with all but the slow KH. PIM was superior to the other cultures but PWM adequately supported good starter growth. Strain SK-11 seemed to be an exception in that it grew best in NDM.

Table 4. Activity test of starters (titrated after 3.5 hr incubation at 37.7 C)

| Culture | Trial | Titratable acidity (%) | | |
|---|---|---|---|---|
| | | I[a] | II[b] | III[c] |
| SK-11 | 1 | .31 | .35 | .35 |
| | 2 | .30 | .32 | .38 |
| | 3 | .32 | .34 | .38 |
| | 4 | .32 | .36 | .37 |
| | Ave. | .313 | .343 | .370 |
| KH | 1 | .32 | .36 | .28 |
| | 2 | .34 | .38 | .29 |
| | 3 | .36 | .42 | .34 |
| | 4 | .35 | .38 | .31 |
| | Ave. | .343 | .385 | .305 |
| R-1 | 1 | .39 | .41 | .36 |
| | 2 | .44 | .45 | .35 |
| | 3 | .49 | .55 | .45 |
| | 4 | .44 | .50 | .40 |
| | Ave. | .440 | .478 | .390 |
| $C_2$ | 1 | .46 | .50 | .37 |
| | 2 | .48 | .52 | .45 |
| | 3 | .55 | .62 | .49 |
| | 4 | .52 | .62 | .48 |
| | Ave. | .503 | .565 | .448 |

Table 4. Continued

| Culture | Trial | Titratable acidity (%) I[a] | II[b] | III[c] |
|---|---|---|---|---|
| $C_6$ | 1 | .43 | .45 | .38 |
| | 2 | .40 | .43 | .36 |
| | 3 | .42 | .52 | .40 |
| | 4 | .48 | .54 | .38 |
| | Ave. | .433 | .485 | .380 |

[a]PWM
[b]PIM
[c]Reconstituted NDM

Bacterial populations in various media

Plate counts were conducted to enumerate starter populations in the three media. KH and $C_2$ strains were selected representing a slow and a fast strain (Table 5). The data indicate growth variation in the three media with the highest population in PIM. Both strains did grow reasonably well in PWM but $C_2$ was more stimulated.

PWM for Cheddar cheese manufacture

Four experimental vats of Cheddar cheese were manufactured using a modified conventional procedure. Commerical mixed freeze dried cultures from D. P. L. Culture Service, were used to prepare bulk starter in PWM and normal whole milk. Both were incubated at room temperature (approximately 25 C) for 12 hr. Behavior of PWM was closely compared with normal

Table 5. Population achieved by lactic streptococci in various media following 18 hr incubation at 21 C

| Organism | Trial | Plate count x $10^8$/ml | | |
|---|---|---|---|---|
| | | I[a] | II[b] | III[c] |
| KH | 1 | 48 | 70 | 43 |
| | 2 | 52 | 78 | 45 |
| | Ave. | 50 | 74 | 44 |
| $C_2$ | 1 | 85 | 121 | 64 |
| | 2 | 94 | 118 | 72 |
| | Ave. | 90 | 120 | 68 |

[a] PWM
[b] PIM
[c] NDM

whole milk starter during cheese making. The finished products were evaluated in a cheese grading class, Food Science and Industries Department, USU.

The cheese making record (Table 6), shows that the acid production in the PWM vat (vats II and IV) was faster from cutting through milling. Higher cooking temperature (104 F) was employed in vat II in an attempt to retard acid development. These results suggest that smaller incoula are also possible with PWM. All cheeses were of good quality (Table 7).

Table 6. Record of manufacture of Cheddar cheese

| Operation | Vat I[a] Time[c] | Temp. (F) | pH | Acid (%) | Vat II[b] Time[c] | Temp. (F) | pH | Acid (%) | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Pasteurization | 30 min | 145 | | .17 | 30 min | 145 | | .17 | Good milk with 3.6 % fat. |
| Added starter | 0:00 | 86 | | | 0:00 | 86 | | 9 | 9 lbs (2%) |
| Added color | 0:01 | 86 | | | 0:01 | 86 | | | 13.5 ml. |
| Added rennet | 0:03 | 88 | | | 0:03 | 88 | | | 40.5 ml single strength |
| Coagulation | 0:19 | 88 | | | 0:17 | 88 | | | |
| Cutting | 0:35 | 88 | | .10 | 0:32 | 88 | | .13 | 1/4" knives |
| Steam on | 0:50 | 88 | | | 0:50 | 88 | | | Slowly by schedule |
| Steam off | 1:20 | 102 | | .11 | 1:20 | 102 | | .15 | Slow agitation |
| Start dripping | 2:20 | 102 | 6.25 | .12 | 2:20 | 104 | 6.20 | .16 | Vat II raised temp. to check acid development |
| End dripping | 2:35 | 102 | 6.15 | .14 | 2:35 | 104 | 6.12 | .19 | |
| Pack | 2:50 | 100 | | .17 | 2:50 | 101 | | .23 | Cut into 8" wide slab |
| Pile two high | 3:35 | 96 | | .24 | 3:35 | 96 | | .30 | |
| Pile three high | 4:05 | 93 | | .30 | 4:05 | 93 | | .36 | |
| Mill | 5:10 | 91 | 5.41 | .45 | 5:05 | 91 | 5.39 | .47 | Wash with 91 F water after milling |
| Salt | 5:20 | | | | 5:15 | | | | 1.24 lbs was used |

| Operation | Vat III[d] Time | Temp. (F) | pH | Acid (%) | Vat IV[e] Time | Temp. (F) | pH | Acid (%) | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Pasteurization | 30 min | 145 | | .17 | 30min | 145 | | .17 | Good milk with 3.5% fat. |
| Added starter | 0:00 | 88 | | | 0:00 | 87 | | | 9 lbs (2%) |
| Added color | 0:00 | 88 | | | 0:00 | 87 | | | 13.5 ml |
| Added rennet | 0:02 | 88 | | | 0:03 | 88 | | | 40.5 ml single strength. |
| Coagulation | 0:15 | 88 | | | 0:15 | 88 | | | |
| Cutting | 0:30 | | | .11 | 0:32 | 88 | | .14 | 1/4" knives. |

Table 6. Continued

| Operation | Vat III[d] Time | Temp. (F) | pH | Acid (%) | Vat IV[e] Time | Temp. (F) | pH | Acid (%) | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Steam on | 0:45 | 88 | | | 0:45 | 88 | | | Slowly by schedule |
| Steam off | 1:15 | 102 | | .12 | 1:15 | 102 | | | Slow agitation |
| Start dipping | 2:15 | 102 | | .14 | 2:15 | 102 | 6.18.17 | | |
| End dipping | 2:30 | 102 | | | 2:32 | 102 | | | |
| Pack | 2:45 | 101 | 6.12 | .20 | 2:45 | 101 | | .23 | Cut into 8" wide slab. |
| Pile two high | 3:10 | 96 | | .25 | 3:10 | 97 | | | |
| Mill | 5:05 | | 5.41 | .45 | 4:50 | | 5.39.48 | | Wash with 90 F water after mill. |
| Salt | 5:10 | | | | 5.00 | | | | 1.24 lbs was used. |

[a]Used whole milk starter pH 4.55, TA 0.83%
[b]Used PWM starter pH 4.50, TA 1,82%,
[c]Time schedules were adjusted to 0:00 at start.
[d]Used whole milk starter pH 4.55, TA 0.84%.
[e]Used PWM starter pH 4.55, TA 1.80%.

Table 7. Composition and grading of finished products

| | Vat I[a] | Vat II[b] | Vat III[c] | Vat IV[d] |
|---|---|---|---|---|
| $H_2O$ (%) | 37.94 | 37.36 | 37.84 | 37.22 |
| pH | 5.10 | 5.12 | 5.08 | 5.14 |
| Fat[e] | 50.1 | 50.9 | 50.7 | 50.8 |
| Texture | slight open | slight open | slight open | good |
| Body | good | slight short | slight curdy | slight curdy |
| Flavor | good | good | slight acid | good |
| Grade | A | A | A | A |

[a]Cheese was made using whole milk starter, graded after one month
[b]Cheese was made using PWM, graded after one month.
[c]Cheese was made using whole milk starter, graded after two weeks.
[d]Cheese was made using PWM, graded after two weeks.
[e]Fat in dry matter.

Costs of starter media

Lagrange and Reinbold (37) reported in a 1968 study of Iowa Cheddar cheese plants that starter costs averaged 1.12 percent of cheese sales income excluding any costs for culture failure. They indicated that culture media constituted about 70 percent of the daily starter cost. Labor contributed another 20 percent, and starter rooms, equipment, seed cultures and other necessary materials made up the balance. These figures reveal the economic importance of culture media to the dairy industry.

Ingredient costs for PWM preparation were computed based upon current prices (Table 8). All the phosphate salts reported by Zottola and Marth (63) are listed. The total ingredients of the most satisfactory blend from an inhibitory standpoint would cost $1.08 (Table 9).

If an operator were to use his own liquid whey, as the goal of this project suggests, the cost would be reduced to $0.73/cwt. The latter figure could range from $0.66 to 0.73/cwt if the least and most expensive blends respectively were prepared.

Commerical PIM was listed from $0.38 to 0.41/lb in March, 1968. The cost was dependent upon the zonal location of the customer and volume purchased. More recent costs were not available but approximately one to two cents/lb increase would be expected. If the 1968 data is used, the cost for PIM would range from $4.48 to 4.84/cwt of starter.

Pretested instant NDM is used in larger volume than PIM. Though this provides no protection against phage attack, it is pretested to assure

Table 8. Price of PWM ingredients in January, 1970

| Ingredient | Volume price quantity | Cost/lb |
|---|---|---|
| | lb | $ |
| Yeast extract | 150 | 1.250 |
| Ardamine Yes[a] | 100 | 1.030 |
| Standard light[b] | 2,000 | 0.050 |
| Whey powder | 2,000 | 0.166 |
| $NaH_2PO_4$ | 2,000 | 0.160 |
| $Na_2HPO_4$ | 2,000 | 0.172 |
| $NH_4H_2PO_4$ | 2,000 | 0.172 |
| $(NH_4)_2HPO_4$ | 2,000 | 0.172 |
| $KH_2PO_4$ | 2,000 | 0.238 |
| $K_2HPO_4$ | 2,000 | 0.308 |

[a]Product of Yeast Products Inc., Paterson, New Jersey.
[b]Product of the Nestle Company, Inc., White Plains, New York.

Table 9. The cost of ingredients of the PWM

| Ingredient | Wt. | Cost |
|---|---|---|
| | lb. | $ |
| Yeast extract | 0.32 | 0.40 |
| $Na_2HPO_4$ | 1.00 | 0.16 |
| $NH_4H2PO_4$ | 1.00 | 0.17 |
| Whey powder | 7.00 | 0.35 |
| Total | 9.32 | 1.08 |

relative freedom from inhibitory substances. Current prices range from $0.28 to 0.30/lb. Ingredients for a ten percent medium would therefore cost between $2.80 and $3.00/cwt.

Marketing costs and profit are difficult to estimate in commerical PIM, however, one might assume that approximately 9.52 percent (11.8 - 0.32 stimulant and - 2.0 phosphate - 9.52 milk whey solids) milk solids is in reconstituted PIM at $0.225/lb (EDW at $0.15 and NDM at $0.30/lb in a 1:1 mixture). This would cost 9.52 x 0.225 = $2.142/cwt for milk solids alone.

An operator making starter utilizing his plant whey by-product and PWM would thus save approximately $2.142 on ingredients, less the receipts he may receive from sale of whey solids. Cheese yield from PIM, however, is superior to PWM because of the casein content. Casein will become a part of the cheese thus increasing yield and return. If PIM is used below the one percent normally used in the vat, the yield will be reduced accordingly. The relative merits of PIM and PWM thus vary with usage levels and composition as well as cost.

If milk is theoretically standardized to equal fat content (i.e., 3.5 percent) after adding one percent starter, the effect of the three media on yield can be estimated. The yield of 37 percent moisture cheese from 100 lb of milk can be calculated using the formula of Van Slyke and Price (56):

(a) Yield of cheese using one percent PWM starter: Assume that milk contains 2.5 percent casein and PWM contains no casein. Though some protein would find its way into cheese as denatured whey protein, this is not included in the calculations.

$$\frac{(0.93 \times 3.5 + 2.5 - 0.1)\,1.09}{1.00 - 0.37} = 9.78 \text{ lb/cwt (cheese yield)}$$

(b) Yield of cheese using one percent PIM starter: Assume that NDM is in PIM at 9.52/2 = 4.76 percent, that NDM contains 36 percent protein and 82 percent of this protein is casein.

4.65 percent x 36 percent x 82 percent = 1.40 percent (casein is reconstituted PIM). One pound of a 1.40 percent casein solution would yield 0.014 lb casein. Total casein content in milk would be 2.5 + 0.014 - 2.514 (percent).

$$\frac{(0.93 \times 3.5 + 2.514 - 0.1)\,1.09}{1.00 - 0.37} = 9.81 \text{ lb/cwt (cheese yield)}$$

(c) Yield of cheese using one percent NDM starter: Assume that NDM contains 36 percent protein and 82 percent of this protein is casein.

10 percent x 82 percent = 2.95 percent (casein in 10 percent reconstituted NDM). One pound of 2.95 percent casein solution would yield 0.0295 lb casein. Total casein content in milk then would be 2.5 + 0.0295 = 2.5295 (percent).

$$\frac{(0.93 \times 3.5 + 2.5295 - 0.1)\,1.09}{1.00 - 0.37} = 9.83 \text{ lb/cwt (cheese yield)}$$

The current cheese price is $0.60/lb. Increased cheese yield would reduce media cost. In this example calculation, PIM increases the yield 0.03 lb cheese/lb culture over PWM. NDM increases the yield 0.05 lb cheese/lb culture over PWM. These figures will return $1.80/cwt culture to the PIM user and $3.00/cwt culture to the NDM user. The net cost for the

media, then, could be calculated (Table 10). It is assumed that packaging, profit, shipping etc., would make PWM cost $1.00/cwt culture.

Table 10. Net cost for three culture media considering cheese yield increases

| | Type of media | | |
|---|---|---|---|
| | PIM | NDM | PWM |
| Initial cost | 4.84 | 3.00 | 1.00 |
| Income from cheese yield increase | 1.80 | 3.00 | ? |
| Net cost | 3.04 | 0.00 | 1.00 |

If PIM produced a yield equivalent to NDM, the net cost would be reduced to $1.84 (4.84 - 3.00). The net cost for phage protection/lb of cheese would be:

PIM: High--$3.04 (net cost)/981 (cheese yield/cwt culture) = 0.310¢,

LOW--$1.84 (net cost)/983 (cheese yield/cwt culture) = 0.187.

PWM: $1.00 (net cost)/978 (cheese yield/cwt culture) = 0.120C

PWM would cost less than PIM, even if the latter produced the same increase in cheese yield as NDM.

NDM is the most profitable medium of the three provided no phage problems develop. A culture service which provides effective culture rotation (for example that available through D. P. L.) (p. 16 of this thesis for address) would be necessary to assure this protection. The cost of such service would vary with the cheese plant volume. It would generally be lower than the cost of PIM or PWM additions. For example, if a plant produced 200,000 lb cheese/month or 10,000 lb/day for 20 days, it would cost only 0.05¢/lb even if the service charge were $100/month.

Effect of variation of yeast concentration on starter activity

The inconsistent results noted on two lots of PIM (page 25 of this thesis) prompted an attempt to provide increased stimulation to PWM. It was hoped this might provide a product as stimulatory as PIM. Two sets of PWM were prepared using 0.32 percent and 0.50 percent yeast extract. Both 16 to 18 hr incubation at 21 C and 16 hr incubation at 29 C were evaluated. The addition of 0.50 percent yeast extract provided slightly more stimulation than 0.32 percent in most cultures but the magnitude of the increase was too low to be significant. The average TA increase of PIM over PIM-1 was 0.11. My results suggest that additional factors are required to provide greater stimuli.

# CONCLUSIONS

1. Whey medium has been improved by phosphate treatment and yeast extract addition.

2. The ability of phosphate treated whey medium (PWM) to stimulate the growth of lactic streptococci and inhibit phage proliferation has been demonstrated.

3. Activity of lactic cultures in PWM starter improved as determined by TA increase, activity test and bacterial population count.

4. A good quality commerical Cheddar cheese can be made using PWM starter.

5. Costs of starter media can be reduced if expensive starter media are replaced by PWM.

6. Further research is required to provide a PWM as stimulatory as the most recent PIM product.

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