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MARY ANN KINDER

Date of Birth — Date de naissance	Country of Birth — Lieu de naissance
30/04/82	CANADA

Permanent Address — Résidence fixe

284 LAGACE, DORVAL, QUEBEC

Title of Thesis — Titre de la thèse

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Causes of Abnormal Starter Culture Development in Yoghurt Formulated from Cottage Cheese Whey

by

© Mary Ann Kinder

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled *Causes of Abnormal Starter Culture Development in Yoghurt Formulated from Cottage Cheese Whey* submitted by Mary Ann Kinder in partial fulfillment of the requirements for the degree of Master of Science in Food Microbiology.

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Supervisor

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Date 19 August 1982

ABSTRACT

Reconstitution of skim milk powder in neutralized cottage cheese whey has been proposed for yoghurt production, as an alternative practice to the customary methods of whey disposal. However, direct incorporation of neutralized whey results in yoghurts of inferior quality with regard to pH and texture. Attempts to improve pH and texture by extending fermentation time from 5 to 8 hours at 42°C were unsuccessful.

Bacteriological analyses revealed abnormalities in the starter culture development, the major effect being an inhibition of *Lactobacillus bulgaricus*. Tests on whey fractions obtained by ultrafiltration identified the permeate as being responsible for the inhibition. Subsequent studies on the permeate components isolated the cause of poor starter culture development to increased levels of whey salts and lactate salts.

Factorial experiments and multiple regression analyses in a broth medium model system confirmed the role of whey salts and lactates in extending both the lag and log phases of growth of the starter culture. An increase of the starter culture inoculum level could reverse the effect on the lag phase but not the effect on the subsequent growth rate. Correction of the abnormalities could be achieved by dilution of the whey to approximately 50% (v/v) with water, prior to use as a reconstituent for the skim milk powder.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1 INTRODUCTION

Like other food processing industries, the dairy industry has followed the general trend toward the consolidation of operations into large multiproduct facilities situated in or close to urban areas (Martin, 1981). Although efficient from the economic and distribution-marketing standpoints, some problems have surfaced, not the least of which is the necessity of disposing of large quantities of the whey resulting from cheese and casein manufacture. Recent figures indicate that in 1978 world production of whey exceeded 88 million tons, of which over 75% was produced by industrialized nations. Canada alone produced approximately 1.2 million tons of whey (Allum, 1980), almost all of which was produced in urban areas.

Most dairy processors can not afford to continue the traditional methods of whey disposal; such as discharge into underground formations (either natural or man made) or agricultural use as fertilizer or stock feed (Ryder, 1980). Instead they have practised disposal into the closest surface water sources or municipal waste treatment facilities. The former method has been essentially discontinued due to increasing environmental concerns, because whey contains about 5% (w/v) of organic material. This organic matter occurs mainly as lactose (4.2 - 4.4%) and whey proteins (0.7%). Whey therefore, has a very high Biological Oxygen Demand (30,000 - 40,000 ppm) (Ryder, 1980) and as such promotes increased microbial degradation activities and associated oxygen loss for considerable distances down stream from the point of discharge.

Whey must be treated to reduce the BOD before disposal. This may be accomplished either in the dairy plant or through the municipal sewage facilities, if they have the necessary capabilities. Some municipalities, such as Mont St. Hilaire, Quebec, insist upon pretreatment, either sedimentation or active aeration to lower the BOD and reduce the strain on their facilties. Other municipalities, such as Edmonton, Alberta, impose a surcharge on the dairies based on the volume and concentration of the effluent to cover the costs of processing.

Dairies which produce large quantities of whey but not enough to make additional treatment economical, are common. Three examples; Lucerne, Northern Alberta Dairy Pool and Palm, are situated in Edmonton, Alberta. Whey release from these plants, primarily from cottage cheese manufacture, averaged 45,000 kg/day, 60,000 kg/wk and 90,000 kg/wk respectively. Furthermore these dairies have no access to either co-operative whey drying facilities or agricultural use.

Direct in-plant use with minimal treatment and capital costs must be considered should surcharges for municipal waste treatment become prohibitive. As energy and labour costs for disposal increase and more criticism is leveled at the wastage of nutrients (FAO, 1974), reevaluation of alternatives will become necessary. Whey is a valuable nutritional resource and attention should turn toward its economic recovery and use. Internationally, many approaches have been instituted or suggested, the range of available uses being greatest in densely populated areas. Some general categories of use for the nutritive components of whey are suggested below, along with their respective advantages and disadvantages.

A. Agricultural Uses of Whey

As a liquid fertilizer applied at a rate of 8 inches per acre, whey has been shown to increase corn yields (Ryder, 1980). This procedure is practised in New Zealand, Pennsylvania and other areas where the costs of transportation of the whey to agricultural land is low and the amount of rainfall is sufficient to prevent a detrimental increase in soil salinity from the whey salts (Kosikowski, 1978; Ryder, 1980). In some countries, such as England, use of whey as fertilizer is prohibited by law (FAO, 1974; Ryder, 1980), presumably because of the general proximity of much arable farming land to recreational and drinking water. Another traditional method of whey utilization has been as an ingredient in livestock feed for pigs, chickens or cows. Even now in the U.K. almost 40% of whey is returned to the farm as livestock feed. This can reduce the costs of feeding animals as the price of whey is lower than most grains with comparable nutrients (Ryder, 1980).

The major drawback is the cost of transporting the whey to the farms. Kosikowski (1978) indicated that for this use to be economically advantageous, the farms must be situated within a 25 mile radius of the cheese plant. This situation occurs more frequently in Europe than in most of North America, hence, in European countries a much larger percentage (up to 90% in Denmark) of the whey produced is returned to farms as livestock feed (Graham *et al.*, 1981).

B. Whey Cheeses

Whey cheeses such as Gjetost, Mysost and Gudbrandsdalsost, are popular in Scandinavia. They are produced by concentrating sweet whey

(pH 5.8 - 6.6) with or without 3.0% fat, depending on the desired quality of the final cheese, to about 84% total solids. The concentrated mixture is subsequently cooled with continuous agitation to promote the formation of fine lactose crystals (Kosikowski, 1978). The resulting product resembles cheese in texture.

Ricotta, an Italian cheese which belongs to another class of whey cheeses, is produced by acid and heat precipitation of the whey proteins and residual caseins. However this preparation procedure does not satisfactorily utilize all of the whey, as the supernatant still has a significant lactose content, and therefore a high BOD (Kosikowski, 1978). Both of these whey cheese types have limited appeal to the North American consumer, although this may be due to very low exposure and an abundance of more familiar dairy products.

C. Whey Beverages

Interest in these products is increasing with beverages such as Rivella (Switzerland and Holland), Bodrost (USSR), whey champagne (Poland) and Whevit (India) already enjoying some success in the marketplaces of the respective countries (Mann, 1972). Most manufacturing procedures consist of clarifying the whey with a subsequent fermentation by either yeasts or lactic acid bacteria and the addition of specific flavours and colours (Mann, 1972; FAO, 1974). Whey in various forms has also been considered for use in soft drinks to increase their nutritive value. Whey can be included in soft drinks as a powder or a protein concentrate, or mixed, ie. ginger ale and frozen whey concentrate in a 3:1 ratio to produce a party drink (Mann, 1974).

D. Whey Powders

This method of recovery is currently the most common in North America. Recent figures (Agriculture Canada, pers. comm.) show that approximately 55% of all whey produced in Canada in 1980 was spray or drum dried, resulting in 56,000 tons of whey powder. Although most of this was used as a stock feed adjunct, the better quality powders produced by spray drying are finding increasing application in human food products as a complete or partial replacement for skim milk powders.

The cost of producing whey powder can be high. FAO figures (1974) indicate that in 1973 it would have cost 1 - 2 million dollars to install drying facilities to handle 500,000 l of whey per day. Furthermore, production costs often reached 11 - 13 cents/kg of whey powder with a sales return of only 11 cents/kg when the amount processed was below 35,000 kg/day. At volumes exceeding 350,000 kg/day production costs dropped to 7 - 9 cents/kg. Thus whey powder production was, and presumably still is, only economical for cheese plants producing very large quantities of whey or a central drying facility located close to several smaller size cheese plants. Obviously, energy costs are a major factor in the drying process.

E. Whey Fractions and Concentrates

To reduce transportation costs, increase the versatility of whey and variety of whey products, many relatively sophisticated concentration and/or fractionation treatments have been applied. Such treatments include ultrafiltration (Maubois, 1978) to remove whey proteins, electrodialysis and ion exhange to remove whey salts

(Houldsworth, 1980), lactose crystallization (Hynd, 1980) and reverse osmosis to concentrate the whey (Kosikowski, 1978). The products of these treatments are becoming of increasing value in the manufacture of infant foods (Mettler, 1980), bakery goods, confectionary and other food items (Holmes, 1979; Mathur and Shahani, 1979; Allum, 1980). However these methods do not always result in complete whey utilization. Disposal of UF whey permeate, salt solutions from dialysis and ion exchange and the mother liquor from lactose crystallization may continue to present a problem. Also, while demand for these products is increasing, production costs are high and may be prohibitive to the dairy with only a moderate whey release (less than 50,000 l per day).

F. A Proposal for Whey Utilization

A proposal that cottage cheese whey be diverted directly into yoghurt production has been proposed by Jelen and Hobal (1974); Hartman (1975); and Jelen (1977). The main idea is that, following neutralization, the whey might replace water or milk as the reconstituent for skim milk powder and cream mixtures prior to normal fermentation procedures. Unfortunately, problems have been encountered in laboratory trials. The authors have independently described inadequate pH development and poor gel strength in the final product. Typical texture and pH measurements are given in Table I. Although to date no research work describing the causes of these abnormalities has been documented, the high final pH would appear to be indicative of impaired starter culture activity.

TABLE I

pH and Texture Measurements
of Whey Yoghurts.

Yoghurt	Reconstitution Medium	pH	Firmness (g/cm^2)
1	Water	4.5	99
2	Neutralized Whey	5.0	42

Incubated for 6 hours at 42°C, 0.1 ml/l inoculum.
(Jelen, pers. comm.)

G. Principles of Yoghurt Production

Two elements are essential for production of good quality yoghurt. These are adequate manufacturing procedures and optimum starter culture development. Each element will be discussed in greater detail.

Manufacturing Procedures

There are three stages that affect texture development in the final yoghurt; standardization of total solids, homogenization and the heat treatment and fermentation (Figure 1).

Yoghurt is normally produced from cows' milk which contains approximately 11.5% total solids (Webb *et al.*, 1978). However to produce a yoghurt with optimum texture development, 14- 18% total solids is preferred (Robinson and Tamime, 1975). This required increase can be achieved by concentration of the milk by reverse osmosis (Jepson, 1977, 1979), by heat evaporation (Wranghede, 1973) or by fortification of the milk by the addition of skim milk powder (Davis, 1973), the last being the most common method (Tamime and Deeth, 1980).

Homogenization redistributes the milk fat and any large skim milk powder particles, if present, uniformly throughout the milk. This produces a homogeneous dispersion of the milk components and will increase the firmness of the final yoghurt, minimizing syneresis (Mulder and Watson, 1974).

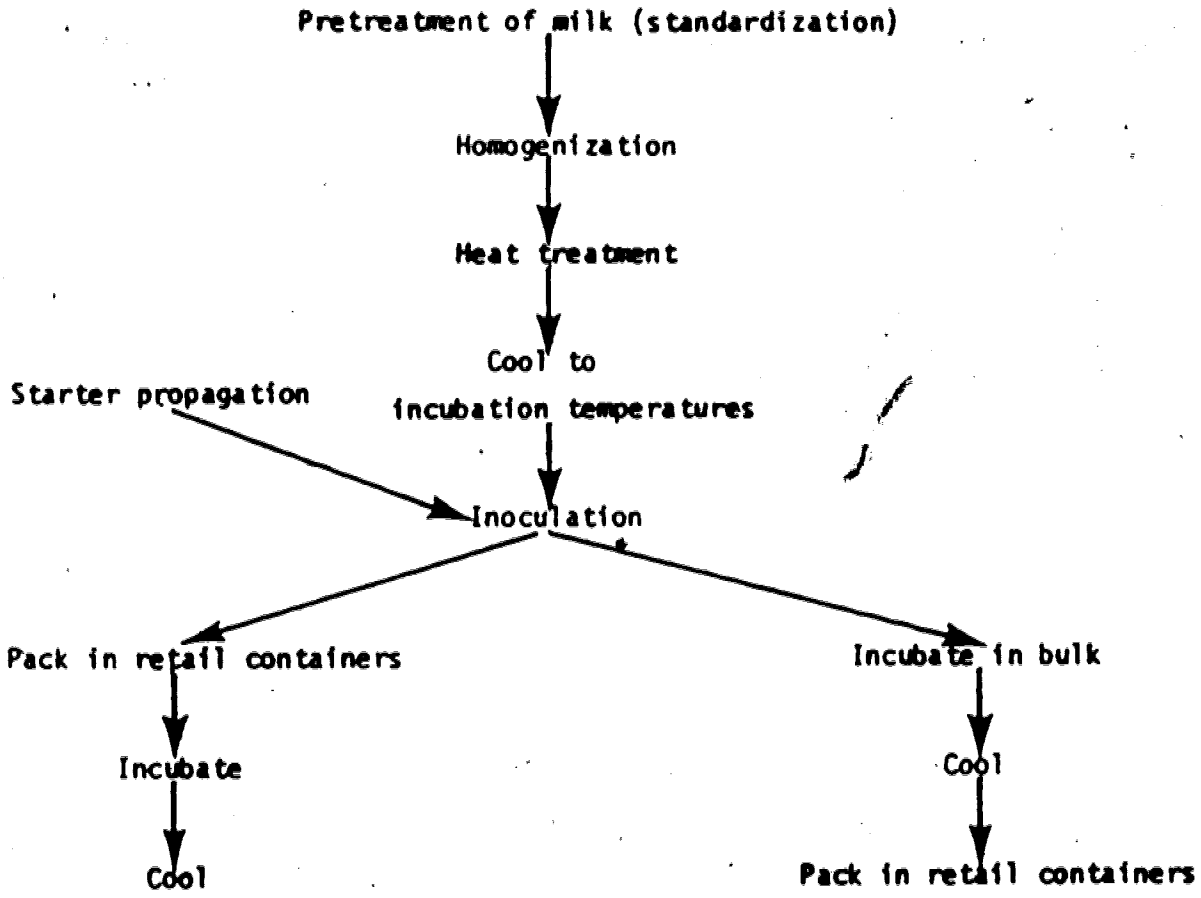

Figure 1.
Industrial production of yoghurt. After: Tamime and Deeth(1980)

Recommended heat treatments vary according to sources (Auclair and Portmann, 1955; Green and Jezeski, 1957; Grigorov, 1966; Feldstein and Westhoff, 1979). A typical commercial procedure is to heat yoghurt formulations to 85 - 90°C for 15 - 30 minutes (Tamime and Deeth, 1980).

This treatment, which like pasteurization reduces the level of milk contamination and increases the milks' suitability as a medium for the starter culture, will also cause specific changes in the milk proteins. According to Webb *et al.* (1978) and Tamime and Deeth (1980) the heat process causes structural changes (denaturation) in the whey proteins. Aggregation of the denatured whey proteins (primarily the β-lactoglobulin) with the κ-casein component of the casein micelles, occurs through the formation of disulfide bonds and an undetermined calcium binding mechanism (Zittle *et al.*, 1962; Webb *et al.*, 1978). Both Kalab *et al.* (1976) and Davies *et al.* (1978) have confirmed this reaction by demonstrating (through electron microscopy studies) the presence of thread-like extensions on the casein micelles following heating.

As a result of these heat induced changes, initial gel formation occurs at pH 5.05 - 5.16 (Tamime and Deeth, 1980; Grigorov, 1966a,b). The production of lactic acid by the starter culture provides the necessary reduction in pH for coagulation to occur.

Starter Culture Development

Starter cultures for yoghurt production consist of approximately equal quantities of two thermophilic species of homofermentative lactic acid bacteria, namely *Streptococcus thermophilus* and

Lactobacillus bulgaricus. Active growth of both types is essential for optimum lactic acid production (Moon and Reinbold, 1976).

It has been determined (Galesloot *et al.*, 1968; Bautista *et al.*, 1966; Hemmé *et al.*, 1981) that *Streptococcus thermophilus* and *Lactobacillus bulgaricus* grow best when propagated together (Figure 2), which results in greater acid production (Table II).

During the initial stages of incubation, *S. thermophilus* can readily ferment the lactose present to create the negative Eh and reduced pH favorable for the lactobacillus (Carr *et al.* 1975, Davis, 1975). The streptococcus also produces formic acid which stimulates the growth of *L. bulgaricus* (Auclair and Portmann, 1959; Veringa *et al.*, 1968).

Though both species are considered to be only weakly proteolytic, *L. bulgaricus* does have the ability to hydrolyze caseins (Tamime and Deeth, 1980). This hydrolysis releases peptides and amino acids which stimulate the growth of *S. thermophilus*. Unfortunately, there is no consensus in the literature as to which amino acids are stimulatory. The numbers suggested range from 2 to 13 and include leucine, lysine, cystine, valine, histidine, aspartic acid, glycine, tyrosine, glutamic acid, isoleucine, methionine, tryptophan and phenylalanine (Bautista *et al.*, 1966; Accolas *et al.*, 1971; Braquart *et al.*, 1978).

The major consideration in selecting cultures is to be certain that the strains of *S. thermophilus* and *L. bulgaricus* chosen are complementary and do not inhibit one another (Hemmé *et al.*, 1981). Both bacterial species produce pyruvate from lactose via the Embden-Meyerhof-Parnas Pathway. Apparently 95 - 98% of the pyruvate is

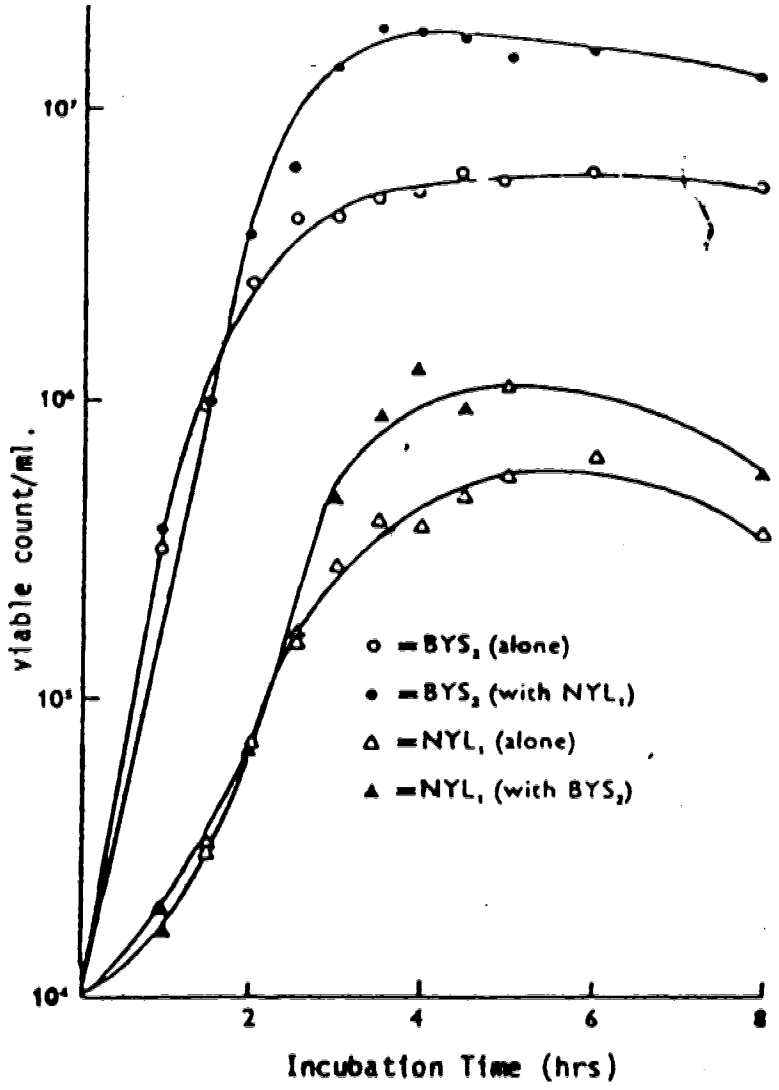

Figure 2
Growth patterns of L. bulgaricus(NYL_1) and S. thermophilus(BYS_2) when propagated alone or in mixed culture.
After: Bautista et al.(1966)

TABLE II

Acid Production by Bacterial Cultures in Milk Propagated Together and Separately.

Culture	% Lactic acid produced		
	propagated separately	propagated separately then mixed	propagated together
Streptococcus thermophilus	0.40		
		0.73	0.94
Lactobacillus bulgaricus	0.24		

1% inoculum incubated at 43°C for 4 hours and 30 minutes.

After: Tramer(1973)

converted to lactic acid while the remaining 2 - 5% is diverted into the formation of cell components with a conseqeuent release of compounds that add distinction to the flavour of yoghurt. These flavour compounds include acetaldehyde, acetoin, acetone and diacetyl (Sandine and Elliker, 1970; Bottazzi *et al.*, 1971).

Effects of Whey Incorporation in Yoghurt Formulations

Reconstitution of skim milk powder in neutralized whey will increase the quantities of lactose, whey protein and whey salts by the concentrations indicated in Table III. This addition alters the conditions of the medium such that growth of the starter culture is affected. For example, if total solids exceed 23% (Tramer, 1973; Pulay and Krasz, 1974) acid production will be reduced. Culture growth is also affected with the streptococcus being slightly inhibited and the lactobacillus severely inhibited. Microscopic examination revealed a high predominance of streptococcus present, while the lactobacillus observed looked slender and distorted (Tramer, 1973).

Another consideration must be the amount of whey salts present in the milk solution. When whey is used as a reconstituent for 15% SMP, the total quantity of whey salts present in the medium will be approximately 2.0%. *S. thermophilus* is known to be relatively salt intolerant, being unable to grow in a medium containing 2.0% NaCl (Buchanan and Gibbons, 1974), which indicates that inhibition of the streptococcus is likely. No specific mention was found in the literature of the salt tolerance of *L. bulgaricus*, however *Lactobacillus*

TABLE III

Typical Composition of Cottage Cheese Whey

Component	Concentration (%w/v)
Total Solids	6.6[a]
Lactose	4.3[a,b]
Protein	0.8[a]
Ash	0.75[c]
Lactic Acid	0.4[d]

a-Tamime and Deeth(1980); b-Josephson et al.(1975); c-Webb et al.(1978); d-Kosikowski(1978).

acidophilus, whose growth pattern is very similar to *L. bulgaricus* can grow in a medium containing 4% NaCl, but not in one containing 6% (Ford *et al.*, 1958). This indicates that the lactobacillus might have a greater salt tolerance that the streptococcus.

Although limited, there is evidence to suggest that the defects, ie. poor texture and abnormal pH development, may be due to abnormal culture development in the presence of the excess whey components. Certainly some physico-chemical effects may contribute to the overall final quality. For example, the balance between the milk protein constituents usually provided for by milk standardization in the normal commercial product may be affected by the addition of whey. This might affect the texture of the final product which is primarily related to the protein content (Tamime and Deeth, 1980).

However, beyond attempts to minimize such effects by maintaining the total solids of trial mixtures in the suggested optimum range, physico-chemical studies were considered beyond the scope of this thesis. Only the microbiological aspects were examined. Two approaches were taken to study the effects of neutralized cottage cheese whey on starter culture development. Initially growth characteristics of *L. bulgaricus* and *S. thermophilus*, acid development and texture were determined in actual yoghurts prepared on a laboratory scale incorporating whey, whey fractions from ultrafiltration, or specific whey components. Subsequently specific factors were studied in a laboratory broth medium model system to gain greater control over variability and permit quantitative evaluation of their influence on culture development.

2. YOGHURT STUDIES

This research consisted of an examination of the effect of whey incorporation on the starter culture growth, acid production and texture of 'set-style' yoghurts. The objectives were to confirm the quality defects observed by Hartman (1975) and Jelen (1977), and evaluate the relationship of these defects to abnormal starter culture development.

Laboratory preparation of the set style yoghurt (Figure 3), a type which undergoes lactic fermentation in the retail containers, was patterned after the industrial process with the notable exception that the homogenization step was instituted after heat treatment. This was necessary to disperse the floc which consistently developed in skim milk formulations containing neutralized whey.

A. Materials and Methods

i. Laboratory Yoghurt Formulations

The basic yoghurt formulation consisted of 15% (w/v) skim milk powder (Northern Alberta Dairy Pool, Edmonton, Alberta), 5% (w/v) cream (35% butter fat) and 80% (w/v) liquid reconstituent. These quantities were chosen due to the constraint that the total solids content should be within the range of 14 - 18% (w/v) (Robinson and Tamime, 1975) and should not exceed 23% (w/v) if culture inhibition is to be prevented (Tramer, 1973). Addition of cream at 5% (w/v) resulted in the production of a low fat yoghurt which contained about 1.5% butter fat.

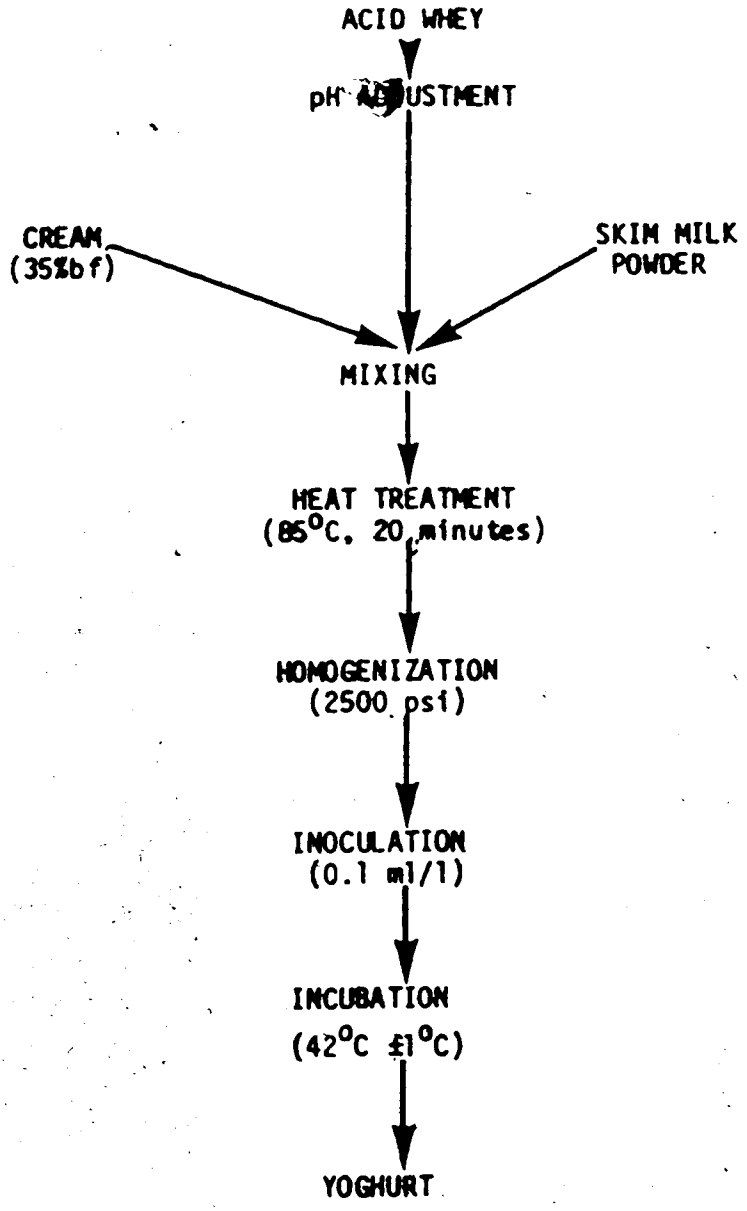

Figure 3. Laboratory procedures for yoghurt production.

Liquid reconstituents

Control yoghurts were prepared using distilled water as the reconstituent. Other reconstituents used in the test yoghurts and their approximate composition, gleaned from published references (Josephson *et al.*, 1975; Kosikowski, 1978; Webb *et al.*, 1978; Tamine and Deeth; 1980), are listed in Table IV. They were prepared as follows:-

Neutralized Fluid Whey

Acid whey from cottage cheese manufacture (pH 4.4 - 4.8) was obtained from a local dairy processor (Northern Alberta Dairy Pool, Edmonton, Alberta) and stored at 4°C upon receipt. The storage period never exceeded 5 days. Prior to use as a reconstituent, the whey was warmed to 20 - 22°C and neutralized to pH 6.7 with a 10 N solution of either NaOH, KOH or NH_4OH depending upon the neutralizing agent under study. The pH change was monitored on a Zeromatic II pH meter fitted with a standard combination electrode (Beckman, Fullerton, California).

Whey Fractions Obtained by Ultrafiltration

Whey, previously warmed to 45°C, was pumped under a pressure of 200 psi through a laboratory ultrafiltration (UF) unit (The Danish Sugar Corporation Ltd., Naskov, Denmark) employing membranes with a pore size of 25,000 daltons. This procedure resulted in two whey fractions, a UF permeate and a UF retentate. Typical UF permeate (pH 4.4 - 4.6) has been reported to contain lactose (4.1%), whey salts (0.75%), lactic acid (0.4% T.A.) and soluble non-protein nitrogen (0.09%)

TABLE IV

Composition of the Reconstituents used in Yoghurt Formulations

Reconstituent	Total Solids (%w/v)	Protein (%w/v)	Lactose (%w/v)	Whey Salts (%w/v)	Lactic Acid (%TA)
Neutralized[a] Fluid Whey	6.6	0.8	4.3	0.75	0.4
UF whey[a] Permeate	5.4	0.0	4.1	0.75	0.4
UF whey[b] Retentate	1.0-4.0	1.0-4.0	-	-	-
Lactose Solution	2.4-4.94	0.0	2.5-5.0	0.0	0.0
Whey Salts Solution	0.33-0.73	0.0	0.0	0.38-0.76	0.0
Lactate Solution	0.43-0.86	0.0	0.0	0.0	9.62[c]

a- After: Tamime and Deeth(1980); Josephson et al.(1975); Kosikowski(1978); Webb et al.(1978).

b- diluted with distilled water to give the desired range of total solids.

c- %w/v, added as 96.2ml/l 1N lactic acid, neutralized with NaOH, KOH or NH_4OH

(Josephson *et al.*, 1975; Kosikowski, 1978; Tamine and Deeth, 1980). The UF retentate (pH 5.3 - 5.5) contained approximately 20% total solids, mostly whey protein. Prior to use as the reconstituent, the permeate was neutralized with 10 N NaOH as detailed for the fluid whey.

Because of the high total solids content of the retentate, it was necessary to dilute it with distilled water before use to obtain the desired solids in the formulation. No neutralization was necessary.

Selected components of UF whey permeate

In order to identify specific effects of each component of the UF whey permeate, it was necessary to test them individually.

Lactose

The effect of lactose was tested at 2.5 and 5.0% (w/v). Solutions were prepared by dissolving the required weight of Bacto-Lactose (Difco) in distilled water prior to use as the reconstituent.

Whey Salts

A dry mixture of whey salts was prepared according to the formula of Jenness and Koops (1962) (Appendix I). Sufficient salt was dissolved to produce concentrations of 0.38 and 0.76% (w/v) and these solutions were subsequently used as the liquid reconstituent.

Lactates

Following preliminary tests to determine the quantity of 1N lactic acid necessary to reduce the pH of 1 l of 10% (w/v) skim milk

from 6.6 to 4.7, 96.2 ml of 1 N lactic acid was neutralized back to pH 6.6 with a 1 N solution of either NaOH, KOH or NH_4OH depending on the lactate under study. The neutralized solution was adjusted to a volume of 1 l prior to use as the reconstituent.

ii. Yoghurt Production

Preinoculation Procedures

The sequence of treatments is outlined in Figure 3. The liquid reconstituent, skim milk powder and cream were mixed together on a heavy duty laboratory magnetic stirrer (Belco Glass Ltd., Vineland, New Jersey) until no undispersed material remained. The mixture was subsequently transferred to a Saf-Guard two gallon home pasteuriser (Schleuter Co., Janesville, Wisconsin) heated to 85°C and held at that temperature for twenty minutes. Following cooling to about 60°C, the solution was passed through a laboratory homogenizer (Gaulin, Everett, Massachusetts) at 2500 psi to disperse and stabilize the milk components. Each homogenized solution was dispersed in 1 l quantities directly into sterile 1 l flasks and further cooled to 46 - 48°C.

Fermentation

Two types of frozen, indirect starter cultures (Christian Hansen Laboratory, Milwaukee, Wisconsin) designated CH-2 and CH-3 were used. Both were provided by the Northern Alberta Dairy Pool (Edmonton, Alberta), and contained equal proportions of strains of *S. thermophilus* and *L. bulgaricus*. Since the cultures could only be stored at -18°C instead of -196°C as recommended, they were replaced every two weeks.

Following removal and thawing of a quantity of frozen culture under aseptic conditions, all flasks were inoculated at the rate of 0.1 ml/l on order to simulate initial commercial starter levels (Toft Jesperson, 1977). Flasks for monitoring bacterial development were incubated directly at 42°C ±1°C for 6 - 8 hours. At the same time, identical inoculated solutions were dispensed in 200 ml quantities into sterile 250 ml beakers. The beakers were then incubated undisturbed under identical conditions, cooled and stored for texture evaluation and total solids determination.

iii. Analyses of the Yoghurts

Bacteriological analyses

A one ml sample was removed immediately after inoculation (0 hour) and at hourly intervals thereafter. Decimal serial dilutions from 10^{-1} to 10^{-8} were then prepared in 0.1% (w/v) peptone blanks according to standard procedures. A 0.1 ml quantity of each dilution was delivered as three separate drops to the surface of prepoured plates of LAB agar (Davis, 1971) (Appendix II) using pasteur pipettes calibrated to deliver 30 drops/ml. To ensure rapid absorption of the drops into the agar and hence minimal spreading, all plates had been previously dried at 50°C for one hour. LAB agar is a medium which permits distinction bewteen the colonies of *S. thermophilus* and *L. bulgaricus* and thus differential enumeration is possible. The respective colony morphologies are illustrated in Plate 1.

To provide optimum growth and maximum colony differentiation, all plates were incubated for forty hours at 42°C ± 1°C under an

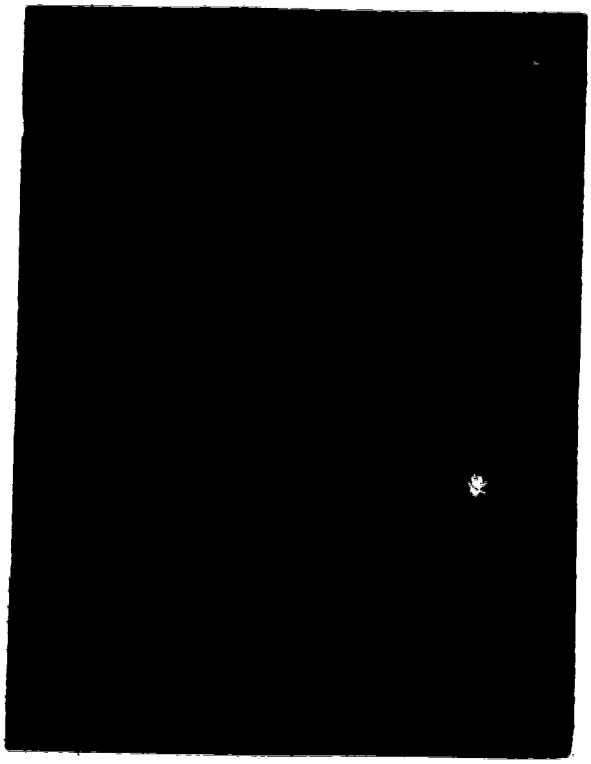

Plate 1.
Colony Morphology of Lactobacillus bulgaricus(large colonies) and Streptococcus thermophilus(small colonies) grown on LAB agar.

atmosphere of CO_2 within BBL anaerobic jars (Becton, Dickinson and Co., Cockeysville, Maryland). While being loaded into the anaerobic jars, a continuous stream of sterile CO_2 was passed over the surface of each plate to eliminate any possible air pockets.

To facilitate distinction between colony types and thus differential enumeration of *S. thermophilus* and *L. bulgaricus*, counting was performed under magnification on a colony counter (New Brunswick, Edison, New Jersey). The number of colony forming units (CFU) per ml of original yoghurt was determined by usual procedures (Marth, 1978).

pH

pH was measured at hourly intervals using a Zeromatic II pH meter with two mini electrodes (Beckman, Fullerton, California).

Total solids

Total solids were determined by the sand pan/vacuum oven method (Marth, 1978). Three to five grams of yoghurt were placed in a tared aluminum dish containing 15 - 20 g of sand. Following mixing and weight determination, samples were dried at 100°C for 5 hours at 4 in. Hg. Dried samples were cooled in a dessicator, reweighed and total solids determined.

Texture

Gel strength was measured on a penetrometer unit (Minarik, Los Angeles, California) equipped with a 12 mm diameter cylindrical probe and attached via a transducer amplifier (Daytronic, Dayton, Ohio) to a

chart recorder (Honeywell, Philadelphia, Pennsylvania). Prior to use the complete unit and all the samples were equilibrated to 10°C for 24 hours in a controlled temperature chamber (Labline, Chicago, Illinois).

The undisturbed 200 ml yoghurt samples, contained in 250 ml beakers, were placed on a weight sensitive disk and the probe was motor driven into the gel. The firmness of the gel was recorded as the force (g/cm^2) required for gel penetration. Three penetrometer readings were taken on each of four replicate samples of all yoghurts produced. The means and standard deviations of the twelve readings were subsequently calculated and subjected to an analysis of variance (Steel and Torrie, 1960) to reveal any significant differences. If significant differences were present, the means were further analysed using Tukey's test (Steel and Torrie, 1960).

B. Results

i. Control Yoghurt

Reconstitution of the basic skim milk powder and cream formulation in distilled water resulted in a yoghurt with acceptable body and acidity, following lactic fermentation by either Hansen CH-2 and CH-3 starter culture. The physico-chemical measurements for the control yoghurt are shown in Table V while the development of the starter culture strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* during fermentation are shown in Figure 4. The pattern of bacterial growth for CH-2 and CH-3 starter cultures compared favourably with published descriptions, in that *S. thermophilus* grew first. Later as the pH and Eh of the medium dropped, growth of *L. bulgaricus* was favoured and by the end of the fermentation period, both *S. thermophilus* and *L. bulgaricus* were present at approximately 10^8 CFU/ml. The decrease in pH associated with bacterial growth induced coagulation of the milk within five hours, comparable to the commercial process.

In subsequent investigations to test the effect of whey and whey components, both the CH-2 and CH-3 starter cultures showed virtually identical response to each particular substrate. Therefore only the results for the CH-2 starter culture are presented in the following descriptions so that clarity could be retained, particularly in the figures.

ii. Whey Yoghurt Formulations

Reconstitution of the skim milk powder and cream in neutralized fluid whey (NFW) confirmed the previous observations of Hartman (1975)

TABLE V

Comparison of SMP/cream Yoghurt Formulations
Reconstituted in NFW* or Water.

Trial	Formulation (%w/v)				Total Solids	Final	Firmness	Coagulation Time
	SMP	Cream	NFW*	Water	(%)	pH	(g/cm^2)	(hours)
Control	15	5	0.0	80	16	4.5	106	5
1	15	5	80	0.0	20	4.9	46	6

* NFW = Neutralized Fluid Whey.

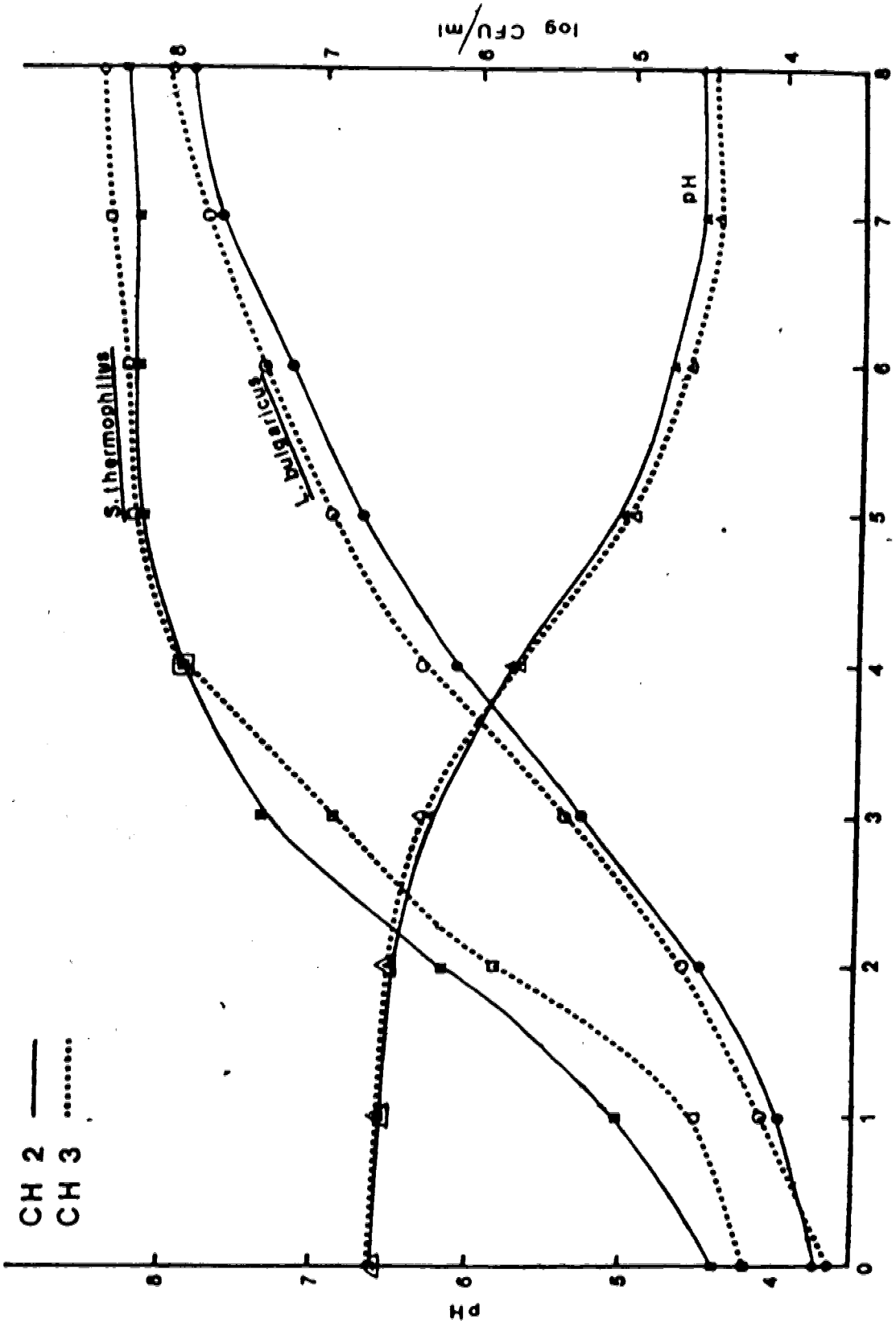

Figure 4. Culture growth and acid production in control yoghurt formulations.

and Jelen (1977). There was abnormal pH development, despite an extension of fermentation time, and a marked decrease in firmness of the final coagulum (Table V, p. 28). Figure 5 illustrates the growth curves of *S. thermophilus* and *L. bulgaricus* during the development of the CH-2 starter culture in whey yoghurt. Comparison with the control Figure 4) reveals only minor effects on the growth of *S. thermophilus*, ie. an increase in time needed to achieve stationary phase and a slight decrease in final cell numbers. However the presence of whey in the formulation has a major effect on growth of *L. bulgaricus*. Though it grows well initially, *L. bulgaricus* showed definite inhibition, reaching stationary phase of 10^6 CFU/ml after 4 - 5 hours of incubation. It was also determined that the inhibition was independent of the alkali used to neutralize the whey. Formulations containing whey neutralized with either NaOH, KOH or NH_4OH produced virtually identical growth curves.

Whey fractions in Yoghurt Formulations

In an attempt to determine the component or components of whey responsible for the observed abnormalities in bacterial growth, further studies were conducted using whey fractions obtained by ultrafiltration (UF) as the reconstituent. The effect of using UF whey permeate as a reconstituent on the texture and pH development of yoghurt and the associated starter culture growth is shown in Table VI and Figure 6, respectively. Again inhibition of *L. bulgaricus* and abnormal pH development was noted. No true coagulation occurred, the final product did not resemble yoghurt, but was more like acidified ropy milk.

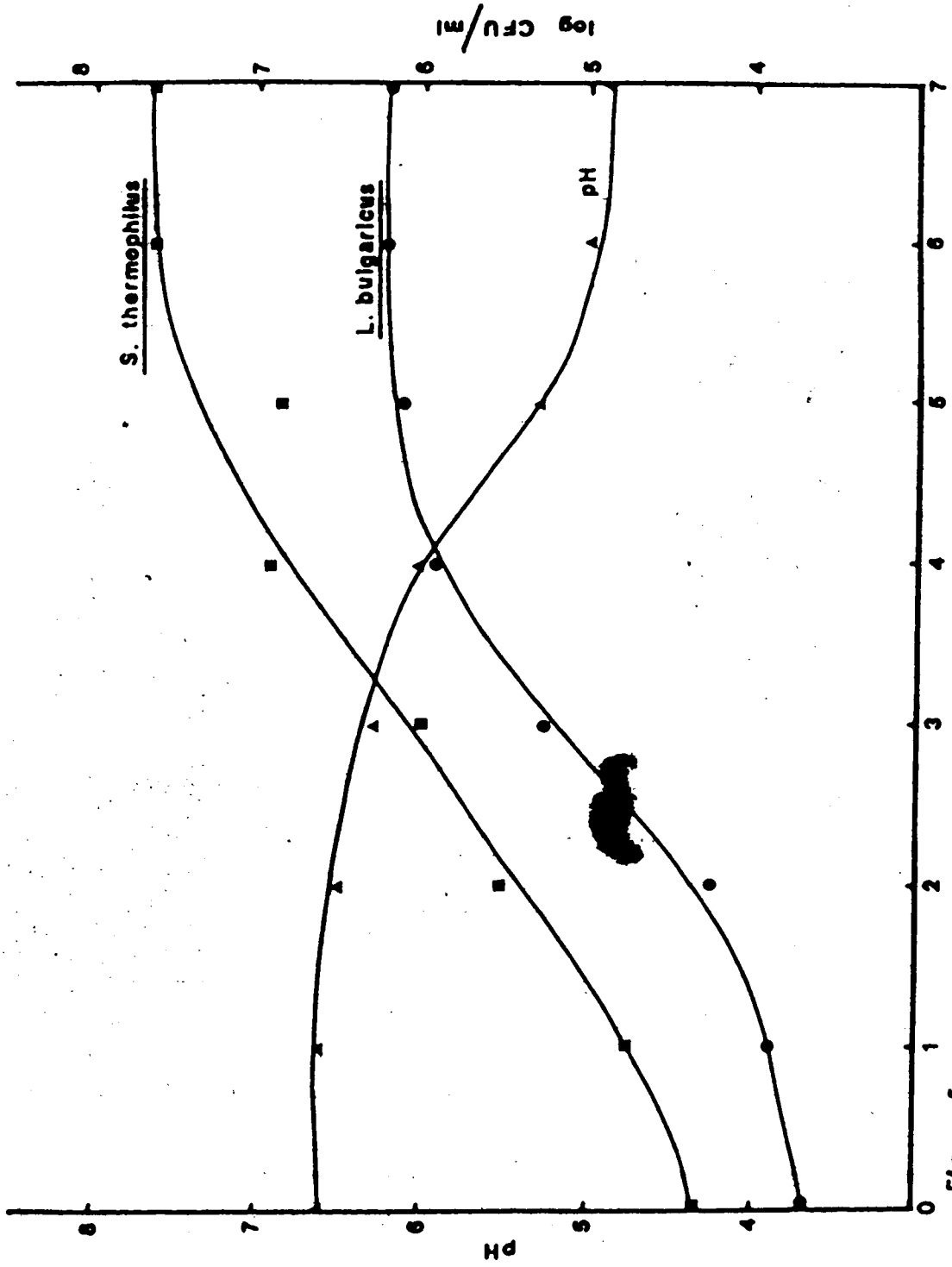

Figure 5. Effect of neutralized fluid whey on growth and acid production of Streptococcus thermophilus and Lactobacillus bulgaricus.

TABLE VI

Comparison of SMP/Cream Yoghurt Formulations
Reconstituted in Water, NFW* or UF Whey Permeate.

Trial	Reconstitution Medium	Total Solids (%)	Final pH	Firmness (g/cm^2)	Coagulation Time (hours)
Control	Water	16	4.5	99	5
1	NFW*	21	4.9	45	6
2	UF Whey Permeate	20	4.9	23	-

* NFW = Neutralized Fluid Whey

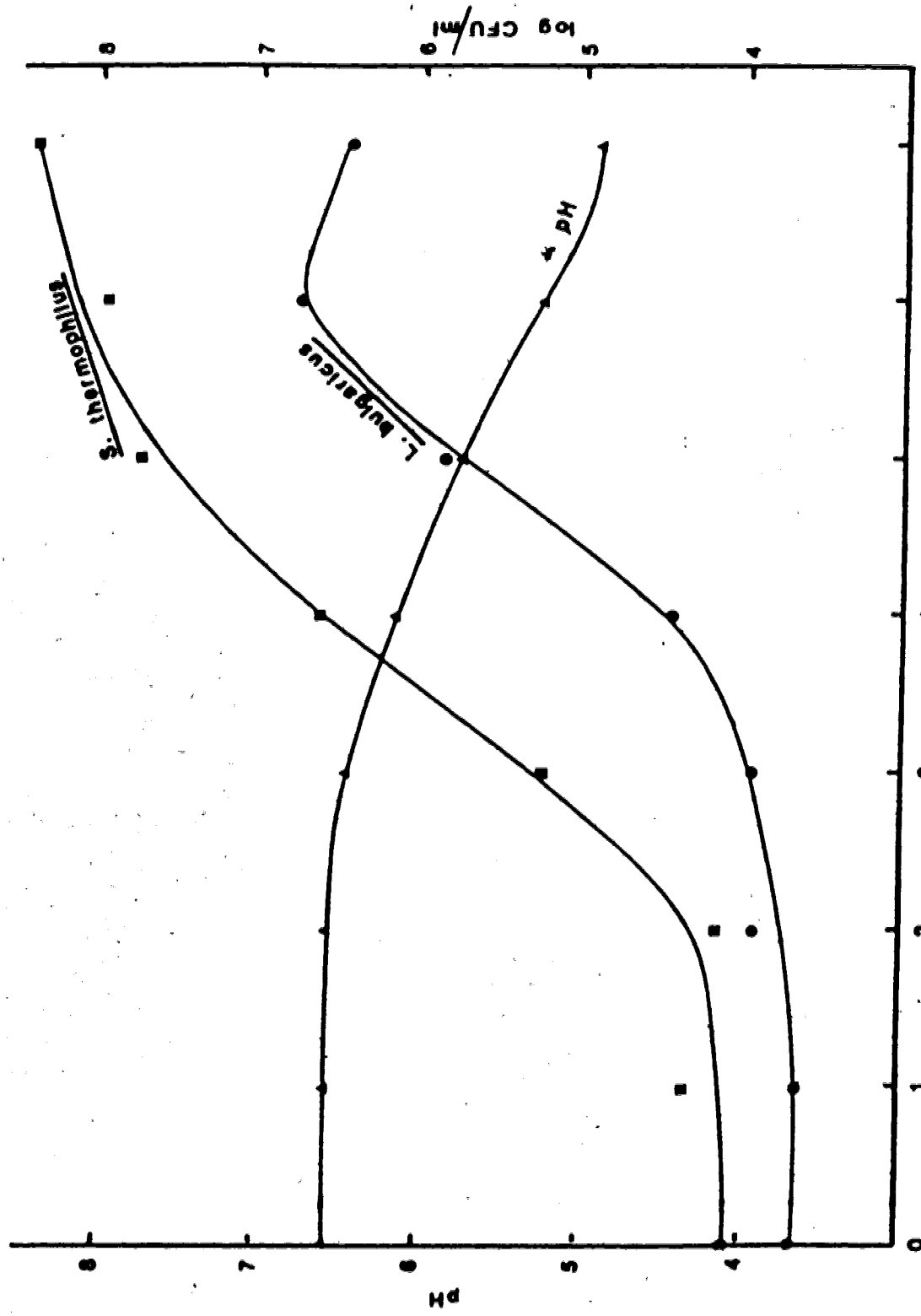

Figure 6.
Effect of utilising UF whey permeate as a reconstituent on growth and acid production of S. thermophilus and L. bulgaricus.

When a dilute UF whey retentate was utilized as the reconstituent, there was a pronounced floc development after the heat treatment, however there were no adverse effects on pH development or yoghurt texture (Table VII). In fact, the yoghurt firmness exceeded the control and was observed to increase as the amount of protein increased. Despite a 22% total solids measurement when 200 ml of UF whey retentate was added, which approaches the recognized inhibitory level of 23% (Tramer, 1973), starter culture development appeared to be normal as indicated by the final pH and coagulation times of the yoghurt. The time consuming data collection on the bacterial profiles was therefore deemed unnecessary.

Having shown that the UF permeate fraction was responsible for culture inhibition, investigations were now directed at the effects of each subcomponent, namely lactose, whey salts and lactates on culture development. Lactose was tested as a commercial compound, whey salts were tested as an artificial mixture, and lactates were tested as lab preparations at concentrations approximating those reported for UF whey permeates (Josephson *et al.*, 1975; Webb *et al.*, 1978; Tamime and Deeth, 1980).

With the addition of lactose no abnormalities were observed in either pH development, texture or coagulation time (Table VIII), or the growth patterns of *S. thermophilus* and *L. bulgaricus* (Figure 7).

When whey salts were included in the formulation at 0.38%, no abnormalities in pH, coagulation time, texture or culture development were noted (Table IX, Figure 8). However, when whey salts were added to the formulation at 0.76% (w/v), examination of starter culture

TABLE VII

Comparison of SMP/Cream Yoghurt Formulations
Containing Increasing Levels of UF Whey Retentate.

Trial	UF Whey[a] Retentate Added (%v/v)	Total Solids (%)	Final pH	Firmness (g/cm^2)	Coagulation Time (hours)
Control[b]	0.0	16	4.6	95	5
1	50ml	17	4.5	165	5
2	100ml	19	4.5	233	5
3	150ml	20	4.4	287	5
4	200ml	22	4.5	311	5

a- Solids content primarily protein

b- SMP reconstituted in distilled water.

TABLE VIII

Comparison of SMP/Cream Yoghurt Formulations
Containing Increasing Levels of Lactose.

Trial	% Lactose[a] Added	Total Solids (%)	Final pH	Firmness[b] (g/cm^2)	Coagulation Time (hours)
Control	0	16	4.6	69	5
1	2.5	18	4.6	72	5
2	5.0	21	4.6	70	5

a - added as Bacto lactose powder(Difco).

b - no significant difference between the means at the 1% level.

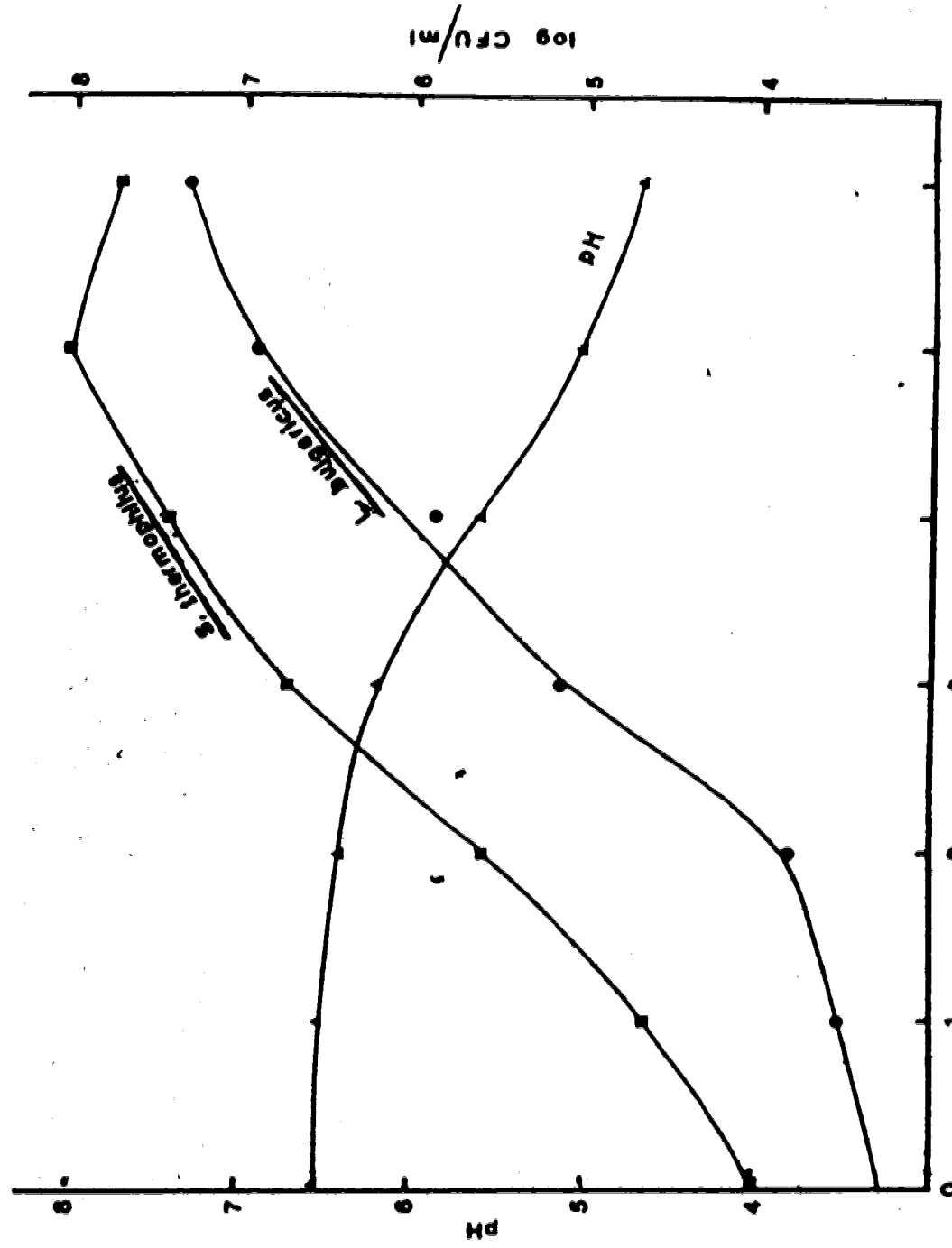

Figure 7.

Effect of utilising a 5.0% lactose solution as a reconstituent on the growth and acid production of S. thermophilus and L. bulgaricus.

TABLE IX

Comparison of SMP/Cream Yoghurt Formulations
Containing Increasing Levels of Whey Salts.

Trial	% Whey Salts[a] Added	Total Solids (%)	Final pH	Firmness (g/cm^2)	Coagulation Time (hours)
Control	0	15	4.6	68	5
1	0.38	16	4.6	71	5
2	0.76	17	4.8	57[b]	8

a - added as an artificial whey salt mixture (Jenness and Koops, 1962)

b - significantly different from the control at the 1% level.

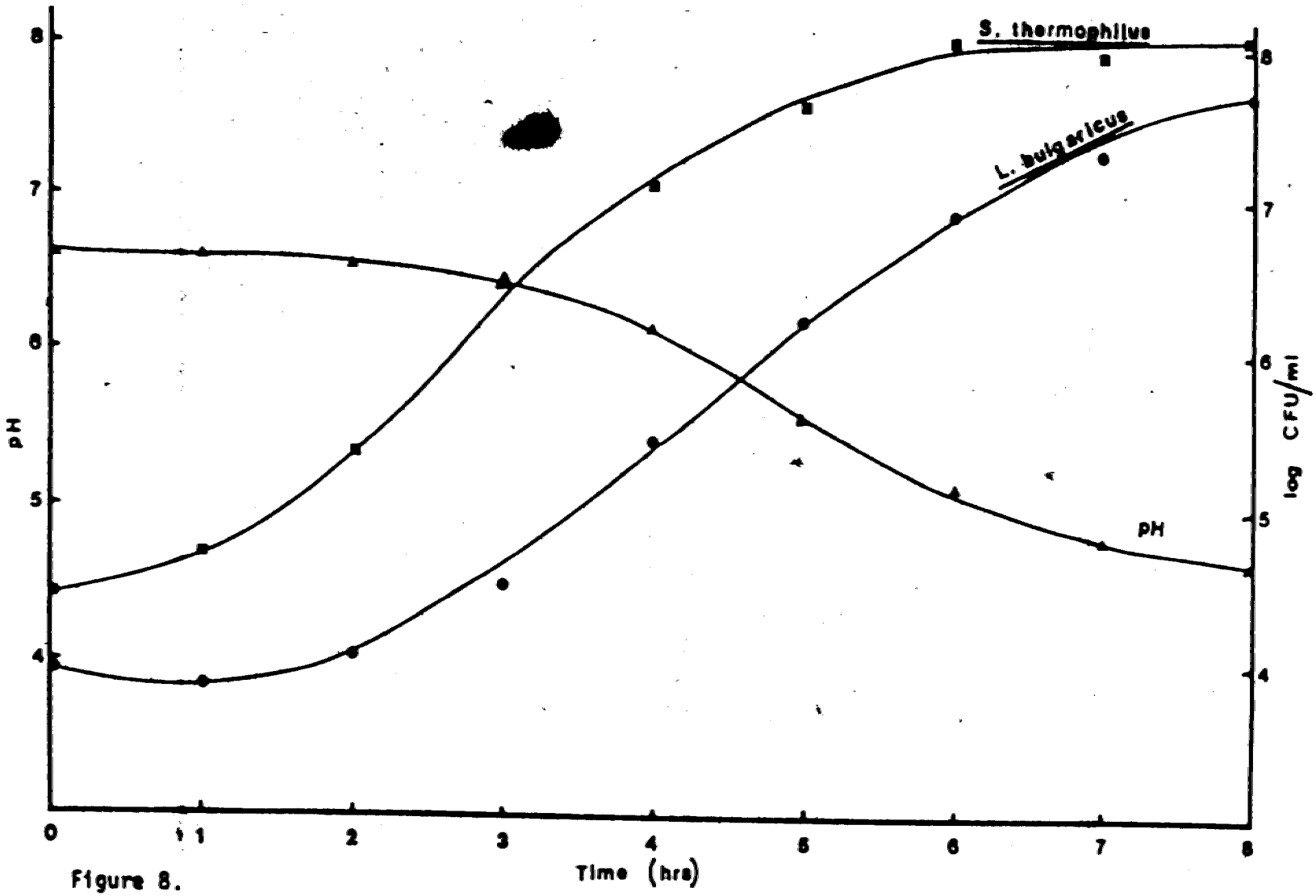

Figure 8.

Effect of utilising a 0.38(%w/v) whey salt solution as a reconstituent on the growth and acid production of S. thermophilus and L. bulgaricus.

growth revealed definite inhibition. The lag phase of both *S. thermophilus* and *L. bulgaricus* was increased while the exponential phase growth rates were decreased (Figure 9). Even with an extended incubation period (8 hours), the final pH, 4.80, was still much higher than the control (Table IX, p. 38).

With the addition of lower quantities of lactates (48.1 ml/l) there was no apparent effect on culture development or yoghurt quality (Table X, Figure 10). However, patterns of culture development and changes in yoghurt characteristics similar to those observed in the presence of whey salts were noted when lactates were added to the formulations at higher levels. Development of *S. thermophilus* and *L. bulgaricus* and pH decrease were severely delayed when the concentration of the individual lactates were equivalent to those present in the MFW, (96.2 ml/l 1 N lactic acid neutralized to pH 6.6 (Table X, Figure 11). These effects, as for neutralized whey, were independent of the alkali used for neutralization (Table X).

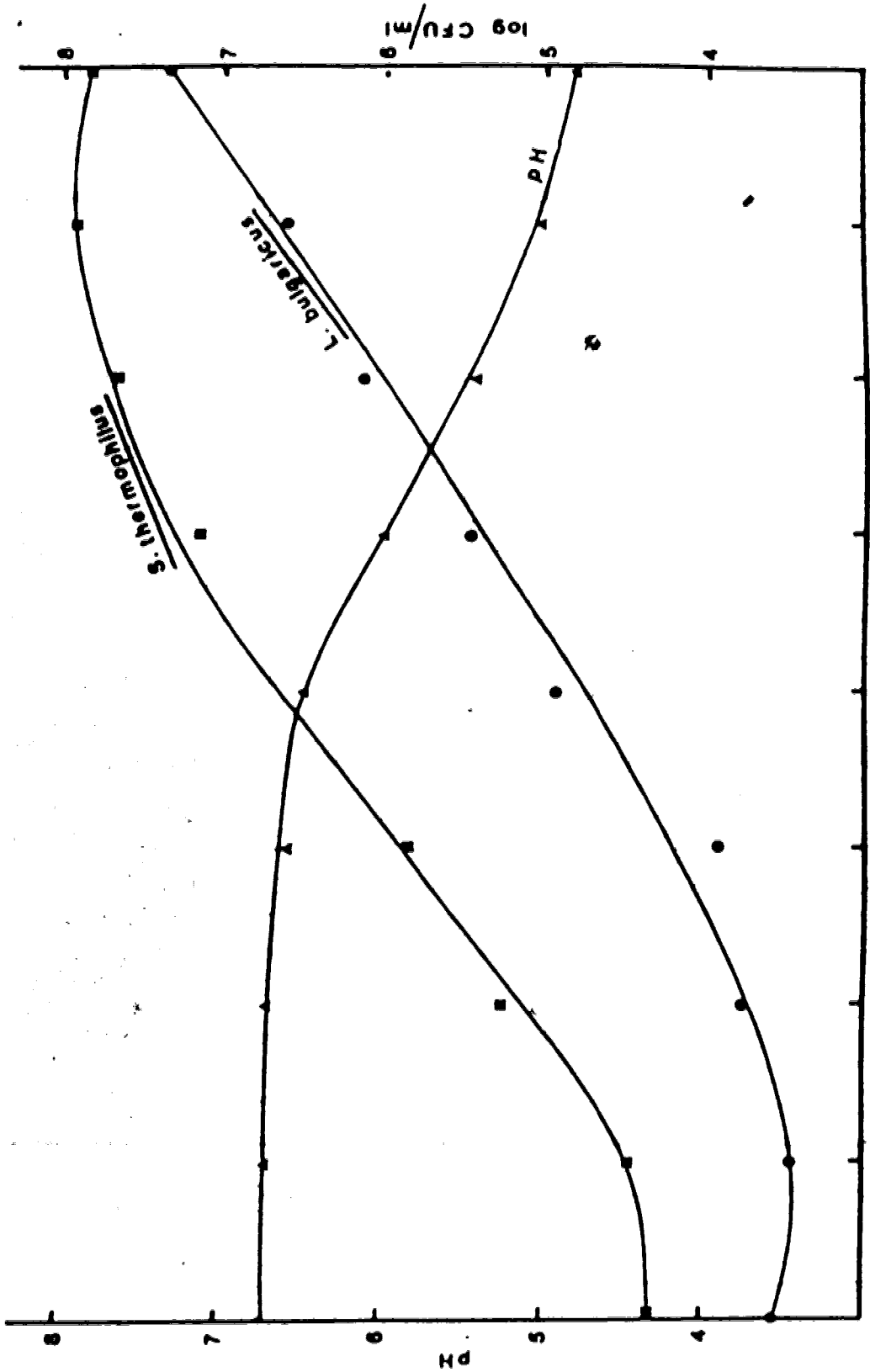

Figure 9.

Effect of utilising a 0.76(%w/v) whey salt solution as a reconstituent on the growth and acid production of S. thermophilus and L. bulgaricus.

TABLE X

Comparison of SMP/Cream Yoghurt Formulations
Containing Various Levels of Sodium, Potassium and Ammonium Lactates.

Trial	Lactate[a] Type	Lactate[a] % added	Total Solids (%)	Final pH	Firmness (g/cm^2)	Coagulation Time (hours)
Control	-	0	16	4.6	68	5
1	Sodium	4.81	16	4.8	73	6
2	Sodium	9.62	16	5.1	56[b]	7
3	Potassium	4.81	16	4.7	70	6
4	Potassium	9.62	16	5.0	60[b]	7
5	Ammonium	4.81	16	4.8	63	6
6	Ammonium	9.62	16	4.9	52[b]	7

a - added as 48.1 or 96.2 ml/l 1N lactic acid, neutralized to pH 6.6 with NaOH, KOH or NH_4OH.

b - significantly different from the control at the 1% level.

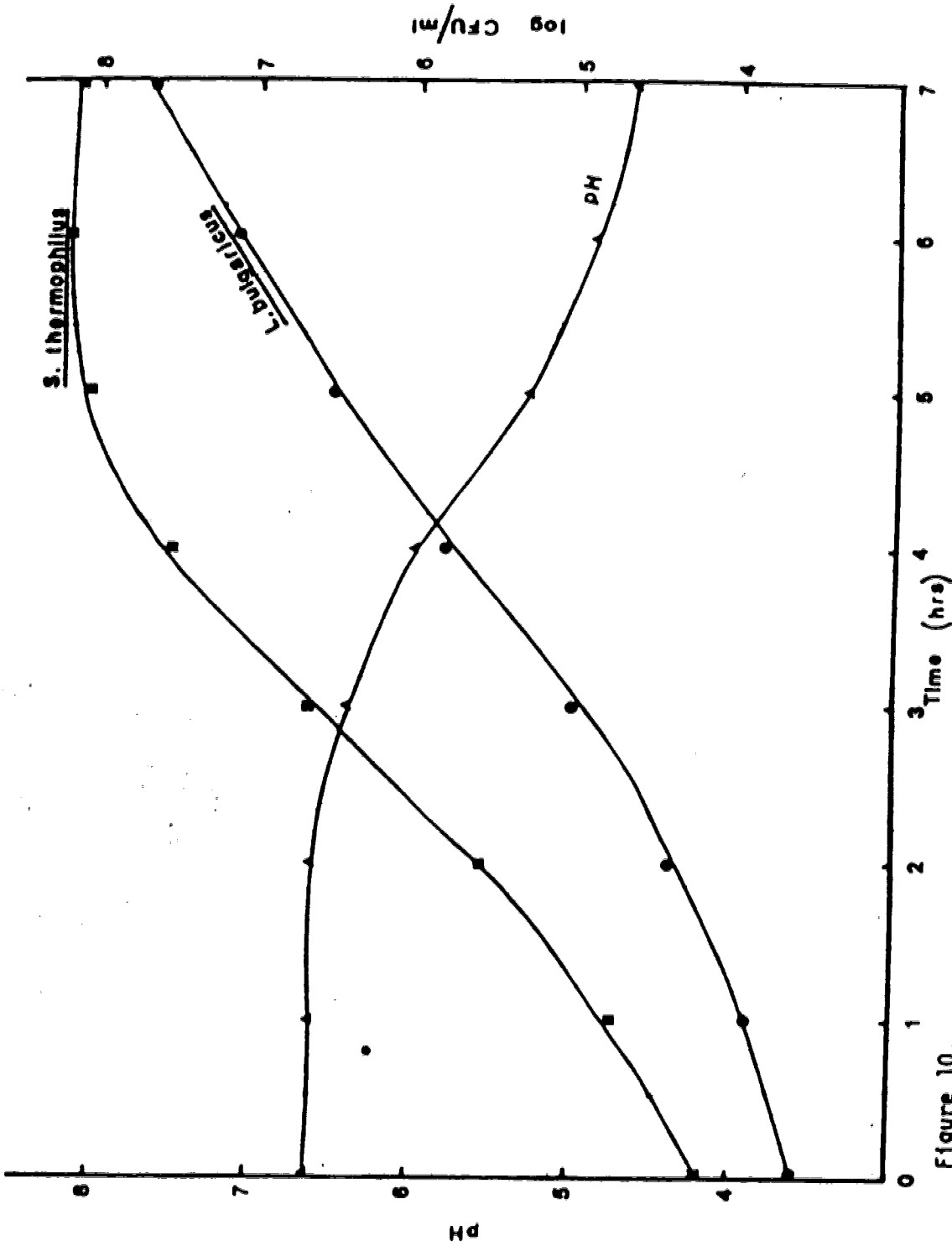

Figure 10.

Effect of utilising a solution containing Sodium Lactate (48.1ml/l) as a reconstituent on the growth and acid production of S. thermophilus and L. bulgaricus.

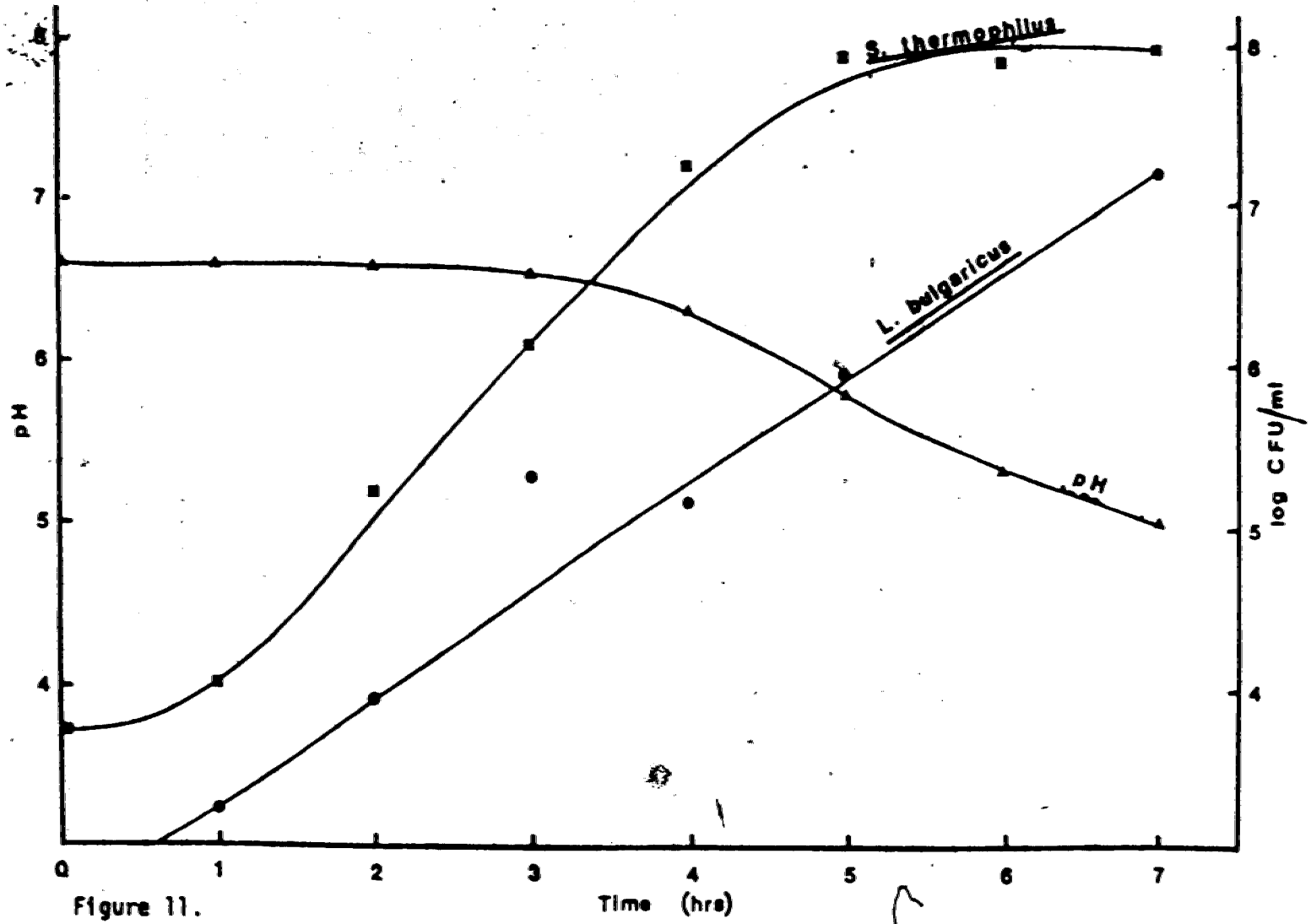

Figure 11.

Effect of utilising a solution of sodium lactate (96.2ml/l) as a reconstituent on the growth and acid production of S. thermophilus and L. bulgaricus.

C. Discussion

The data presented above confirm the results of Hartman (1975) and Jelen (1977) that incorporation of neutralized fluid whey in yoghurt formulations produces yoghurts with weak texture and poor pH development. The results also revealed the inhibitory action of whey on the growth of the yoghurt starter cultures, primarily the growth of *L. bulgaricus*. This inhibition also occurred when UF whey permeate (containing lactose, whey salts and other low molecular weight compounds) was used as the reconstituent, while use of UF retentate (which contains the higher molecular weight compounds) had no effect on starter development. When the individual UF whey permeate components were examined, lactose produced no inhibition whereas the presence of whey salts or lactates had a definite inhibitory effect on culture growth. In addition to the inhibitory action of the UF permeate, whey salts and lactates, the yoghurt exhibited weak texture and poor pH development. This indicates that the cause of both abnormal starter culture development and weak texture is due to alteration in the ionic strength of the milk formulation.

Texture also depends on the types and proportions of proteins present in the formulation (Tamime and Deeth, 1980). The results of addition of extra whey proteins in the yoghurt formulation shows that the poor texture which occurs with the addition of whey can be remedied by increasing the whey proteins. However, in the presence of excess whey proteins, a floc develops after the heat treatment. This causes problems in both cleaning procedures and post pasteurization contamination. The floc was eliminated by the addition of extra

sodium caseinate, but the growth of the starter culture was not measured in those preliminary tests. In any case, this extra step would defeat the purpose of direct whey utilization with minimal treatment and costs.

3. FACTORIAL DESIGN STUDIES

The yoghurt study identified whey salts and the lactates resulting from neutralization as the whey components responsible for poor culture development, particularly *L. bulgaricus*. However the cumbersome nature of the yoghurt based studies (lengthy preparation time and extensive experimental manipulations and resources), prevented examination of a number of factors simultaneously. Only one variable could be studied at a time and as such any complementary effects or interactions among factors and the environmental conditions could not be detected. Furthermore, the numerous stages of experimentation greatly increased the chances of introducing variability unrelated to the factors under study.

A simplified and flexible system incorporating a modified broth medium and optical density measurements was therefore developed. This increased the control of the environmental conditions and permitted quantitation of the primary effects and interactions of added whey components on culture growth.

Following the selection of a clear broth medium which would support starter culture growth comparable to that obtained in milk formulations, a 2^k complete factorial experimental design (where k = numbers of variables to be studied at 2 experimental levels) was chosen as the appropriate approach to the simultaneous evaluation of the effects of inhibitory whey components and possible counteractive factors on culture development. Subsequently, the generation of regression equations describing the quantitative relationship between

whey parameters and culture growth, enabled assessment of possible courses of action to remedy the abnormalities.

A. Materials and Methods

1. The Factorial Design

The preceding studies indicated that the ultrafiltration whey permeate, whey salts as an artificial mixture (Jenness and Koops, 1962), and lactates as a laboratory preparation were the components inhibitory to starter culture development. Since lactose was independently eliminated as a contributory factor and the various lactates were determined to have similar effect, only whey salts and sodium lactate were incorporated into the factorial design. In addition, in recognition of the fact that relatively high cell numbers can tolerate or adapt more quickly to adverse environmental conditions than low numbers, culture inoculation level was included as a possible positive influence.

In all cases, experimental levels were chosen to relate to the concentrations normally encountered in yoghurt milks formulated with whey. The factors and levels employed are described in Table XI. In each case the intervals between the test levels were deliberately chosen for linearity to conform to the required assumptions of the anticipated first order regression model. Furthermore, to expedite statistical analysis, variable levels were coded so that the lower level was equivalent to '-1' and the upper level to '+1'. Base levels

TABLE XI

Levels of Factors Used in the 2^3 Factorial Design

Level	Sodium[a] Lactate (g/100ml)	Whey[b] Salts (g/100ml)	Inoculum[c] Level (CFU/ml)
high	0.9	1.05	3.8×10^4
base	0.5	0.7	2.0×10^4
low	0.1	0.35	2.0×10^3

a - added as a commercial product (Baker Chemical Co.,N.J.)

b - added as an artificial whey salts mixture (Jenness & Koops,1962).

c - using a Redi-Set CH-2 yoghurt starter culture

were designated as '0'. Coding and decoding (when required) was achieved by application of the following formula (Bacon and Henson, 1971).

$$\pm 1 = \frac{\text{actual concentration} - 1/2\ (\text{high level} + \text{low level})}{1/2\ (\text{high level} - \text{low level})}$$

For example to code for the high '+1' level of whey salts, 1.05 g/100 ml)

$$\frac{1.05 - 1/2\ (1.05 + 0.35}{1/2\ (1.05 - 0.35)} = \frac{1.05 - 0.7}{0.35} = \frac{0.35}{0.35} = +1$$

The layout of the 2^3 complete factorial design is detailed in Table XII. The effects of all combinations of levels of all factors on culture growth can be observed and analysed by the appropriate statistical procedures from the data obtained on growth in the 12 experimental units.

ii. Basic Growth Media

The commonly available broth media for culturing lactic acid bacteria are listed in Table XIII. Most were deemed unsuitable for use either because of significant salt content (which would interfere with whey salt assessment), for example, MRS and TJB, or because following lab tests they were found to be incapable of supporting yoghurt culture growth comparable to that encountered in milk formulations, for example, LBS and PM. Peptonised milk, fortified with 0.3% (w/v) yeast extract (PMYE), pH 6.6, was finally selected as the base medium. It

TABLE XII

2^3 Factorial Design

Flask	Sodium Lactate (X_1)	Whey Salts (X_2)	Inoculum Level (X_3)
1	-1	-1	-1
2	+1	-1	-1
3	-1	+1	-1
4	+1	+1	-1
5	-1	-1	+1
6	+1	-1	+1
7	-1	+1	+1
8	+1	+1	+1
9	0	0	0
10	0	0	0
11	0	0	0
12	0	0	0

Sodium Lactate: +1 = 0.9; 0 = 0.5; -1 = 0.1 (g/100ml).
Whey Salts: +1 = 1.05; 0 = 0.7; -1 = 0.35 (g/100ml).
Inoculum Levels: +1 = 3.8×10^4; 0 = 2.0×10^4; -1 = 2.0×10^3 (CFU/ml).

TABLE XIII

Commercial Media Available for
Culture of Lactic Acid Bacteria.

Media	Producer
MRS: deMann,Rogosa & Sharpe	Difco
LBS: Lactobacillus Selection Broth	Becton, Dickinson &Co.
TJB: Tomato Juice Broth	Difco
PM: Peptonised Milk	Difco
PMYE:Peptonised Milk-Yeast Extract	Difco

provided the clarity necessary for optical density measurements of culture growth and contained no extraneous salts.

The PMYE medium was prepared according to standard procedures and dispensed into 250 ml Erlenmeyer Flasks which had been adapted by the addition of a Spectronic 20 tube as a side arm (Plate 2). The volume of medium per flask varied from 48.0 to 49.0 ml depending on the experimental run and required volume of inoculum. Flasks were prepared in duplicate, one serving as a culture growth unit, the other as an uninoculated OD control.

Addition of Whey Salts

A dry mixture of whey salts was prepared according to the method of Jenness and Koops (1962) as detailed in Appendix I. Coded levels, -1, 0 and +1 (Table XII) were obtained by the addition of 0.35% 0.7%, and 1.05% (w/v) of the dry salt mixture respectively to the appropriate flasks.

Addition of Sodium Lactate

Sodium lactate was added from a commercial solution (Baker Chemical Co., Phillipsburg, New Jersey) containing 60% Na Lactate. The coded levels, -1, 0 and +1, were obtained by addition of 0.1%, 0.5% and 0.9% (w/v) respectively.

After addition of both whey salts and sodium lactate, the flasks were gently heated to dissolve the whey salts and then sterilized at 121°C for 15 minutes.

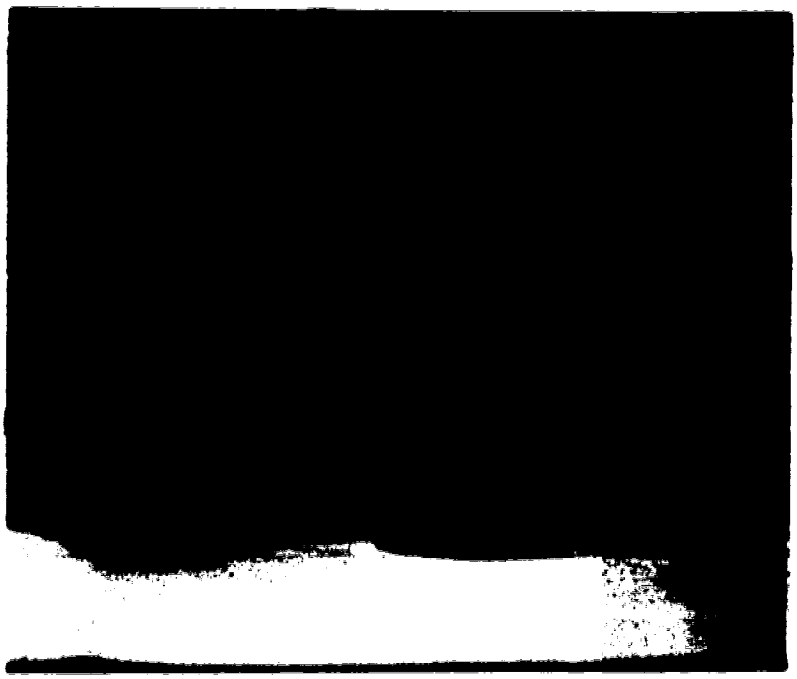

Plate 2.

An example of the flasks used in the 2^3 Factorial Design Experiments.

Preparation of Cultures and Flask Inoculation

Since the previous studies demonstrated the virtually identical response of both the CH-2 and CH-3 starter cultures (Christian Hansen Laboratory, Milwaukee, Wisconsin), this study used only Redi Set, CH-2.

A standard plate count (SPC) using LAB agar and incubation at 42°C for forty hours under a CO_2 atmosphere resulted in a count of 9.6×10^8 CFU/ml for frozen Redi Set culture. One ml quantities of defrosted starter culture were diluted in 0.1% (w/v) peptone blanks to yield stock solutions, approximately 10^5 and 10^6 CFU/ml respectively. From these stock solutions, either 1.05 ml or 2.0 ml quantities were used as inoculum. For example Flask I, (Table XII, p. 51), required a low level of inoculum, therefore 1.05 ml of the 10^5 CFU/ml stock solution was used. However for flask 8 (Table XII, p. 51), requires a high level of inoculum, therefore 2.0 ml of the 10^6 CFU/ml stock solution was used.

iii. Culture Growth

Culture development was monitored by observing the changes in optical density. A spectrophotometer set at 600 nm was used because the media had its lowest absorbance at this wavelength. Growth curves were determined by plotting absorbance, taken at inoculation (0 hour) and at thirty minute intervals thereafter, versus time. The two parameters of culture growth used to examine the effects of each factor were lag period and growth rate. The end of the lag period was identified by the change in OD_{600}/30 minutes being greater than

0.03 units and the growth rate was measured as the slope of the curve during the exponential phase of growth.

These parameters were measured for each growth curve and the results were subsequently analyzed through a computer assisted standard regression analysis.

iv. Further Tests

Positive effects suggested by the factorial design studies were examined in the model system to determine their influence. These positive effects were also tested in laboratory scale yoghurt production to confirm the results.

B. Results and Discussion of the Factorial Design Studies

Examples of the growth curves obtained for the Hansen CH-2 starter culture in a number of the experimental runs comprising the 2^3 factorial design are shown in Figure 12. Variation in both the lag phase and subsequent growth rate was used in the statistical description of the effect of sodium lactate (X1), whey salts (X2) and inoculum level (X3). Details of all measurements obtained are shown in Table XIV. Regression analysis of the lag phase measurements resulted in the following equation

$$Y_1 = 3.7 + 3.28X1 + 2.28X2 - 0.066X3$$

Similar statistical analysis of the variations in culture growth rate (Table XIV) gave the following regression equation.

$$Y_2 = 2.3 - 1.44X1 - 0.40X2 + 0.006X3$$

Using the Student's two tailed t test, all regression coefficients were significant ($P \leq 0.05$, 8 d.f.). Interactions between the factors were not examined because the coefficients of determination (R^2) were, in both cases, significant ($P \leq 0.05$, 8.d.f.). This indicated that the equations, Y_1 and Y_2, contain all the major effects.

The signs of the regression coefficients indicate that the lag phase (Y1) will increase with increasing concentrations of lactates (X1) and whey salts (X2) and decrease as inoculum levels (X3) increase. The inverse is true for the growth rate (Y2) which will increase with a decrease in the amounts of whey salts and lactates present and increase with the inoculum level. Hence, only increases in inoculum level could be used in attempts to counteract the abnormalities observed in starter culture development in neutralized fluid whey yoghurts.

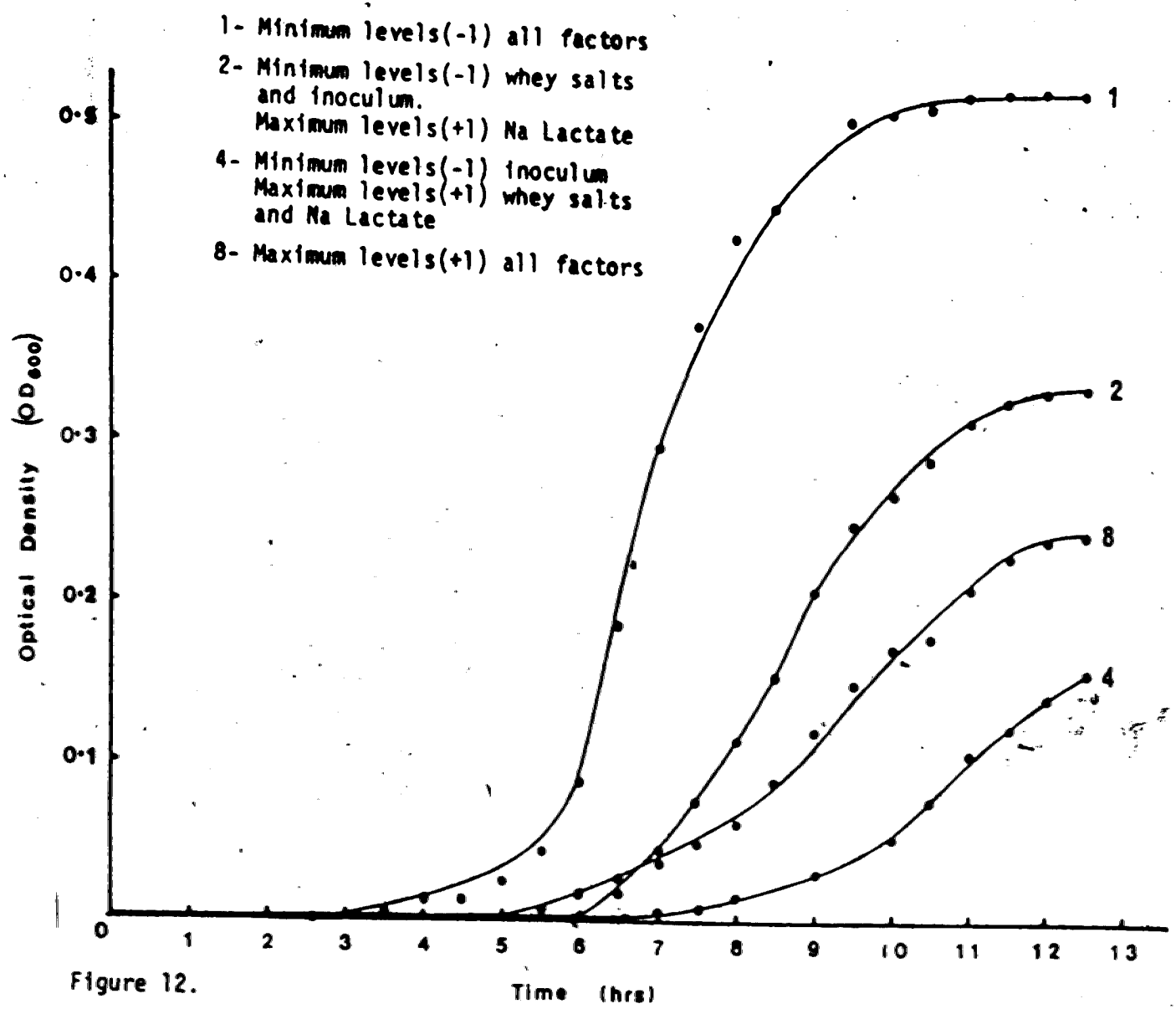

Figure 12.

Examples of growth curves, obtained from flasks containing specific mixtures of sodium lactate, whey salts and inoculum levels, used in the factorial design.

TABLE XIV

Effects of the Combinations of Factors on the Lag Period and the Exponetial Growth Rate of the Starter Culture, Redi Set, CH-2.

Flask	Lag Period (Hours) (Y_1)	Growth Rate (Y_2) $\frac{OD_{600}}{Time}$
1	5.5	2.05
2	7	0.91
3	6.5	1.60
4	11.5	0.51
5	4	2.22
6	4.5	1.25
7	4.5	1.91
8	8	0.50
9	5	1.43
10	5	1.33
11	5	1.40
12	5	1.45

i. Results of tests of the predictions determined from the regression equations.

The comparison between the predicted lag period obtained from the regression equation and the actual experimental values obtained from the increased levels of inoculum are shown in Table XV. The corresponding regression line is illustrated in Figure 13. Good agreement was found up to inoculum counts of 77 x 10^3 CFU/ml. The subsequent divergence at higher inoculum counts was attributed to the inability of the regression equation to account for the necessary period of adaptation (approximately 3 hours) upon the transfer of the diluted starter culture directly into the PMYE growth medium (Control, Table XV). Such an adaptation was not as pronounced upon transfer of the frozen culture into the control yoghurt formulations (Figure 4, p. 29).

The results suggest that reversal of the abnormalities of lag phase might be achieved by addition of approximately 5 times normal inoculum to NFW yoghurt formulations. However, increasing the inoculum level is unlikely to affect a substantial change in the growth rate as indicated by the value of the coefficient of the inoculum level (X3). This was confirmed by comparison of the predicted and experimental growth rates shown in Table XVI and the regression line for inoculum (Figure 14). Thus although a higher inoculum might be expected to reverse the log phase abnormalities in NFW yoghurts, it would appear to have no substantial effect on the subsequent abnormal culture development.

TABLE XV

Predicted and Experimental Values of the Lag Period of CH-2 Culture in Peptonised Milk-Yeast Extract Media

Trial	Volume[a] Used	CFU/ml ($\times 10^3$)	Predicted Lag Period	Experimental Lag period
1 *	1 ml	19.2	6hr. 59min.	7 hr.
2 *	3 ml	57.6	4hr. 28min.	4hr. 30min.
3 *	4 ml	76.8	3hr. 11min.	4 hr.
4 *	5 ml	96.0	1hr. 55min.	3hr. 45min.
5 *	7 ml	134.0	0 hr.	3hr. 45min.
6 *	10 ml	192.0	0 hr.	3hr. 30min.
control [b]	3 ml	57.6	-	3hr. 15min.
control [b]	5 ml	96.0	-	3 hr.

* contains 0.9g/100ml sodium lactate and 0.7g/100ml whey salts.

a- volume of stock solution used as an inoculum

b- PMYE only, contains no salts.

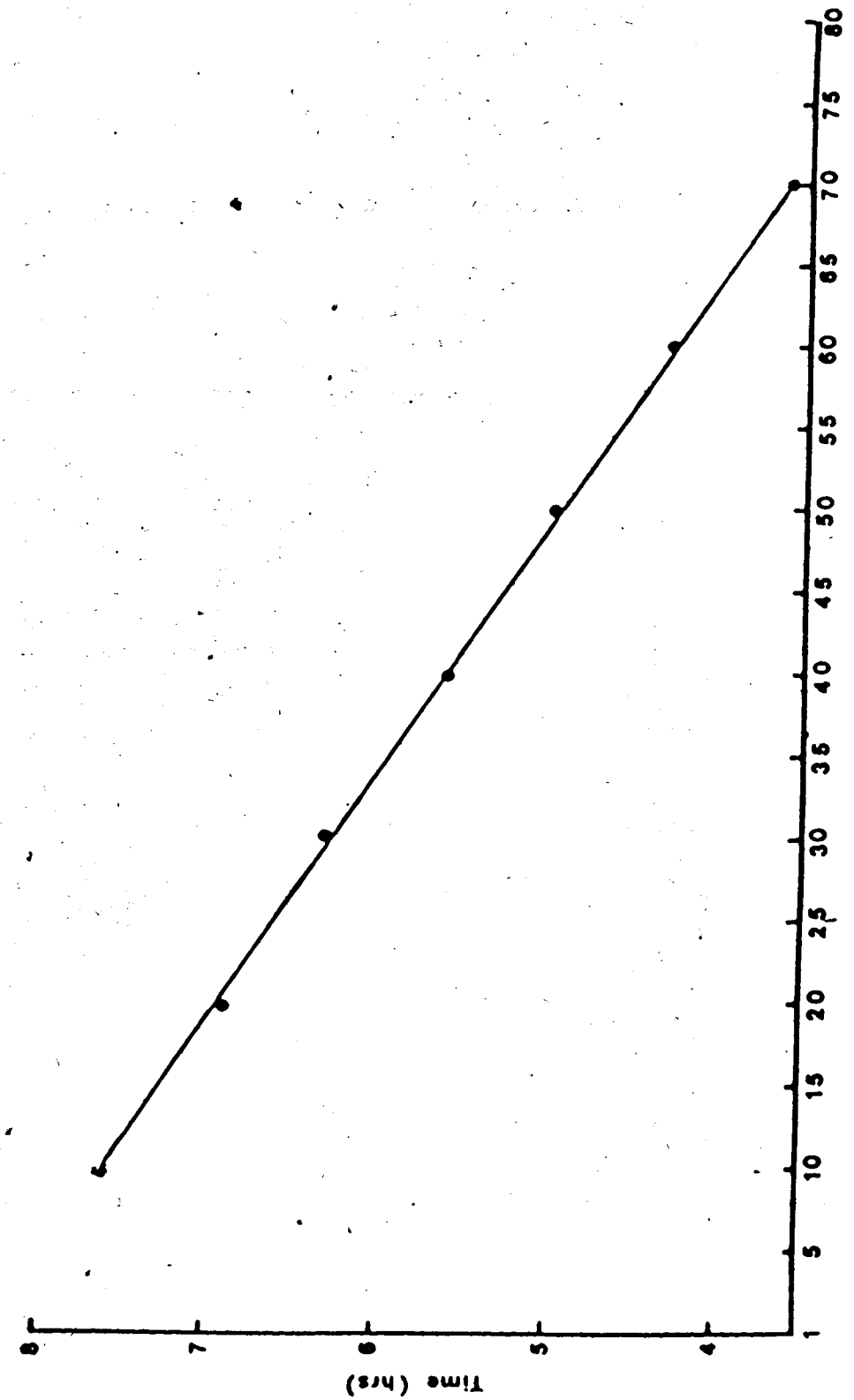

Figure 13. Regression line for lag period of Redi Set starter culture CH-2, with increasing levels of inoculum and constant levels of other factors.

TABLE XVI

Predicted and Experimental Values of the Exponential Growth Rate of CH-2 Culture in Peptonised Milk-Yeast Extract Medium.

Trial	Volume[a] Used	CFU/ml ($\times 10^3$)	Predicted Growth Rate (ΔOD/Δtime)	Experimental Growth Rate (ΔOD/Δtime)
1*	1 ml	19.2	0.84	0.79
2*	3 ml	57.6	1.08	1.07
3*	4 ml	76.8	1.19	1.19
4*	5 ml	96.0	1.31	1.29
5*	7 ml	134.0	1.53	1.25
6*	10 ml	192.0	1.88	1.34
control[b]	3 ml	57.6	-	2.00
control[b]	3 ml	96.0	-	2.10

* contains 0.9g/100ml sodium lactate and 0.7g/100ml whey salts.

a- volume of stock solution used as an inoculum

b- PMYE only, contains no salts.

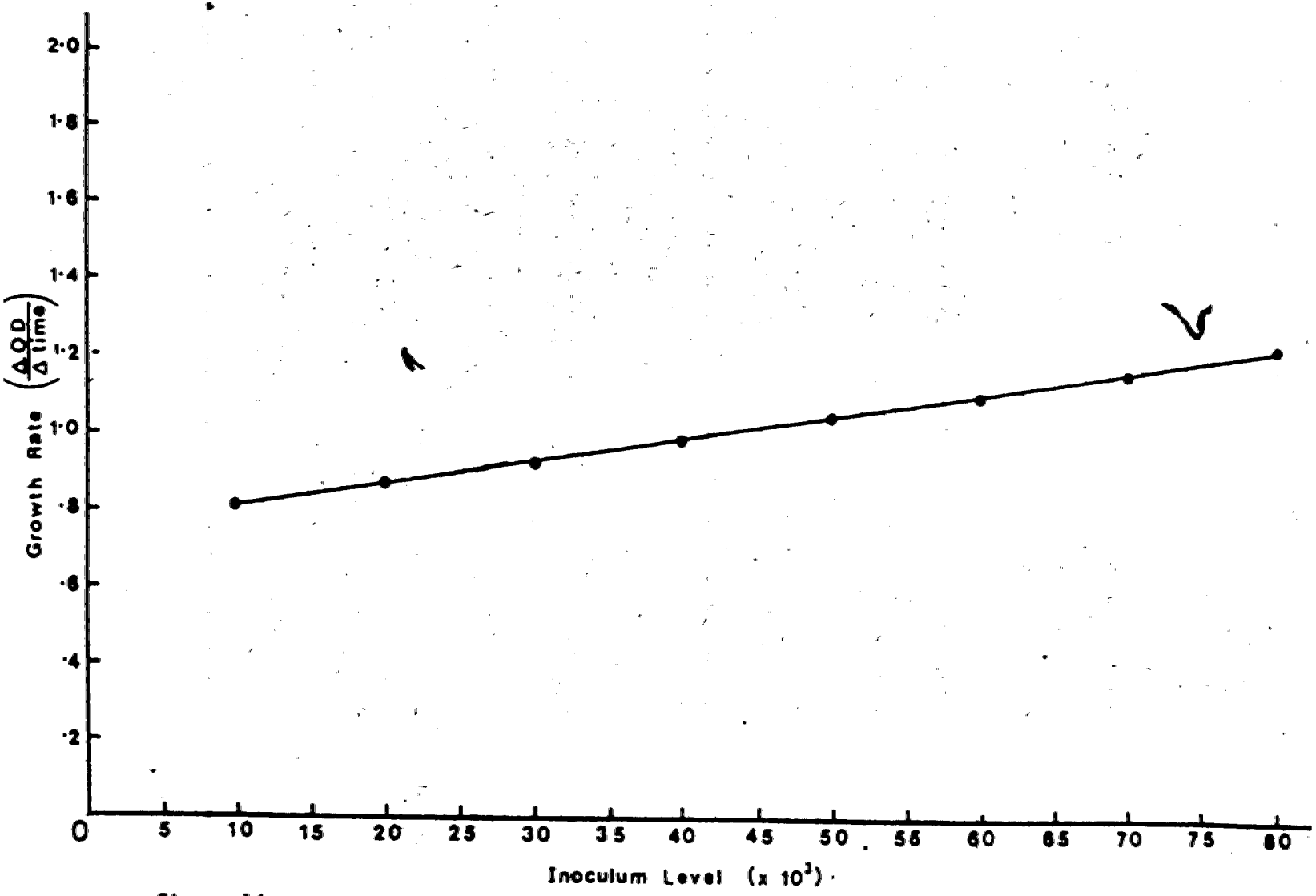

Figure 14.

Regression line for the exponetial growth rate of the Redi Set starter culture CH-2, with increasing levels of inoculum and constant levels of the other factors.

ii. Test with Diluted Neutralized Fluid Whey.

As increasing the inoculum does not prevent abnormal culture growth, the alternative solution would be to decrease the concentration of whey salts and lactates. This would be achievable in NFW yoghurts by introducing prior dilution of the NFW reconstituent. In order to test this possibility, a 50% dilution of NFW in distilled water was used as the reconstituent. The use of 50% (v/v) NFW resulted in the yoghurt texture and starter culture development shown in Tale XVII and Figure 15 respectively. All observations compared favourably with those obtained for the control yoghurt formulated in water (Figure 4, p. 29). Also at this concentration of whey, there was no problem with floc development following the preparatory heat treatment stage as there was in the 100% NFW formulations. This eliminated the need to reverse the order of homogenization and heat treatment, as discussed previously. Although no trials were conducted to evaluate subtle increases above 50% in the NFW concentration, it is suggested that there would be only a slight gain in the overall incorporation of whey before either abnormalities in culture development and/or floc formation would be observed.

TABLE XVII

Comparison of SMP/Cream Yoghurt Formulations
Reconstituted in 50% NFW* or Water.

Trial	Reconstituting Medium	Total Solids (%)	Final pH	Firmness[a] (g/cm^2)	Coagulation Time (hours)
1	Water	16	4.5	50	5
2	50% NFW* 50% Water	18	4.6	49	5

* NFW = Neutralized Fluid Whey

a - no significant difference between the means at the 1% level.

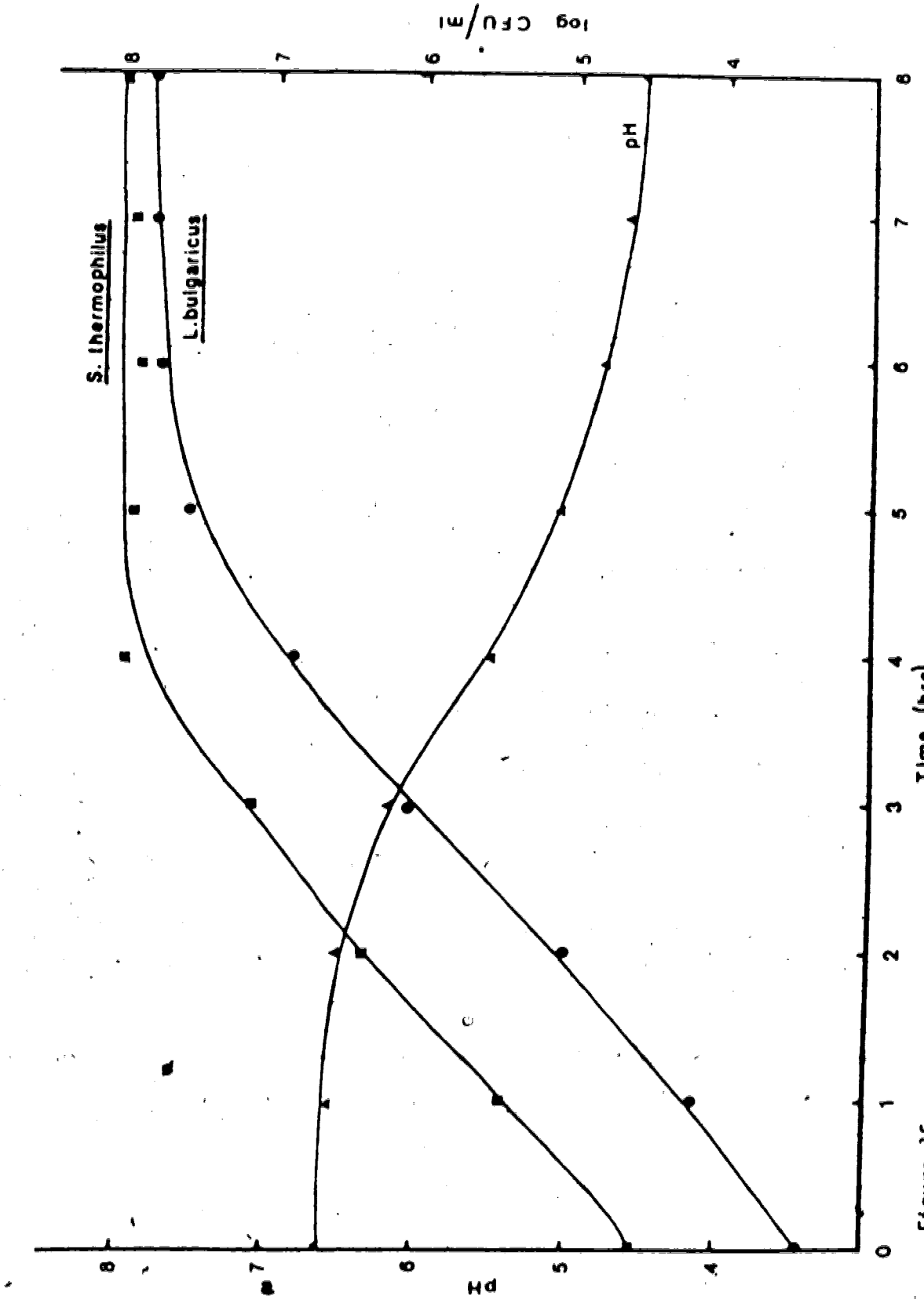

Figure 15.
Effect of utilising a reconstituent containing 50% neutralized whey/50% water on the growth and acid production of S. thermophilus and L. bulgaricus.

4. CONCLUSIONS

In this thesis, the effects of incorporation of neutralized cottage cheese whey into yoghurt formulations were examined. The three major effects are the development of weak texture, abnormal acid production and inhibition of the yoghurt starter culture, primarily *Lactobacillus bulgaricus*, the major acid producing species. Both the weak texture and the inhibition of the culture growth are due to the increased quantities of whey salts and lactates, which increased the ionic strength of the formulations.

The inhibitory action of the whey salts on culture development was confirmed by both laboratory yoghurt studies and factorial design experiments. However, further study on the effects of salts on *L. bulgaricus* is necessary to determine if the inhibition was due to a specific salt or the total salt concentrations present. Remedial action suggested by both the yoghurt studies and the factorial design experiments is a decrease in the amount of whey salts and lactates present. Using a 50% (v/v) dilution of whey in a set style yoghurt formulation produced a good quality yoghurt with no floc formation or abnormal culture growth.

Other possibilities that could be examined are the use of stabilisers to improve texture, or use of whey-yoghurt formulations in 'stir-style' yoghurts because the softer gel is more cohesive and is therefore less susceptible to mechanical damage.

This work has indicated that although the amount of cottage cheese whey that could be channeled into set-style yoghurt production

is less than originally proposed, it would still be a viable method for whey utilization in plants with moderate tandem production of cottage cheese and yoghurt and limited capital resources for new equipment installation. Further studies of all possibilities are necessary, as well as sensory and market evaluations of the whey yoghurts.

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6. APPENDIX I

5.1 Preparation of Whey Salt Solutions

KH_2PO_4	15.80g
$K_3Citrate.H_2O$	5.08g
$Na_3Citrate.5H_2O$	1.20g
K_2SO_4	1.80g
$CaCl_2.2H_2O$	13.20g
$Mg_3Citrate.H_2O$	5.02g
K_2CO_3	3.00g
KCl	10.78g

Grind each salt in a mortar and mix thoroughly in a dry blender. This quantity is sufficient for ten liters. For one liter weigh out 7.59g, dissolve in 975-990 ml of distilled water, adjust pH to 6.6 with 1.0-1.5N KOH and make to one liter.

For the artificial system 0.76g were used per 100ml of media. The pH was adjusted after autoclaving to 6.6 if necessary but was normally found to be very close without adjustment.

7. APPENDIX II

6.1 Preparation of LAB Agar

Lactose	20g
Tryptone	10g
Beef Extract	10g
Yeast Extract	10g
Tomato Juice (fresh)	25ml
K_2HPO_4	2g
Tween 80	1 drop
Agar	15g
Water	

Tomato juice was prepared by blending fresh tomatoes in a Waring blender until a fine puree was formed. The puree was the centrifuged (10 minutes, 8000rpm) to separate the pulp and the seeds from the juice. The juice was decanted and used immediately in the LAB media. The pH was adjusted to 6.5, if necessary, and the media was then sterilised at 121°C for 15 minutes.