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CHEMICAL PRESERVATION OF WHEY AND ITS UTILIZATION BY YEAST FERMENTATION

BY

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ABSTRACT

The chemical treatment of whey to prevent deterioration during short storage, and suitability of chemically preserved whey for propagation of Torula cremoris, and Saccharomyces fragilis were investigated.

Treatment of whey at pH 3.5 - 4.4, with 0.3% sodium sulfite, or 0.15% sodium metabisulfite, or 250 p.p.m. of hydrogen peroxide was found to extend the storage life of whey up to 3 weeks, when stored at 70 - 72°F under sterile conditions.

Treatment with sodium sulfite and sodium metabisulfite rendered the whey unsuitable for yeast propagation. The whey, chemically preserved by 200-250 p.p.m. hydrogen peroxide, when treated with catalase then followed by heat treatment at 70°C for 5 minutes, was suitable for the growth of the yeast species. The propagation of yeast was able to reduce the dissolved chemical oxygen demand (C.O.D.) of whey by about 75%, with the lactose utilization efficiency being 70-72%.

Preservation of whey by hydrogen peroxide, and utilization for yeast production is very promising. The system has real merit as a means of coping with the problems of whey disposal.

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INTRODUCTION

Whey, a by-product of cheese manufacture, is of increasing importance to the dairy industry. High biological and chemical oxygen demand (B.O.D. and C.O.D.) of whey makes its disposal difficult and costly. Whey is an excellent medium for the growth of microorganisms. Unless it is treated, it cannot be discharged into water ways or small sewage systems, for reasons of overloading and pollution.

Porges (1958) reported that 100 lbs. of skim milk solids in an analogous situation of dairy waste, required all the oxygen dissolved (8.4 p.p.m. at 25°C) in almost 12 million imperial gallons of water (Wasserman, 1960 a). Since the chemical oxygen demand (C.O.D.) of whey is 40 - 50 times greater than that of average dairy waste, and the biological oxygen demand (B.O.D.) of whey is as high as 100 times stronger a polluting agent than that of domestic sewage (Burdock et al., 1967), the needs for waste treatment and the difficulty in treating it by means of natural aeration, can be appreciated.

Economic Utilization

The simplest method of utilization or disposal of whey, is to return it to the farm where it can be used as livestock feed, but this is feasible only in certain

areas and only to a certain extent (Wasserman, 1960 a). Several methods for utilization of whey are in use. Some of the whey is dried or further processed. Investigations have been carried out by many workers, and reviewed by Webb et al. (1948) on the production of ethyl alcohol and riboflavin by fermentation of whey. Conversion of whey to yeast protein has also been considered in the past by Graham et al. (1953), and Wasserman (1960). In spite of the above methods, it was estimated that in the U.S.A. in 1959, only 29% of the whey was utilized (Porges, 1959), and the remaining about 70% portion was still discarded as a waste because of the impracticability of economic utilization.

Chemical Treatment

The natural microflora already present in whey may very easily cause its spoilage by undesirable fermentations and thereby deplete the original lactose content of whey. Such deteriorative changes may occur during transportation from factory to the farm and even during short storage at room conditions in the factory or at the farm. Further, the need to handle such huge quantities, (approximately 850 gallons of whey for every 1,000 gallons of milk used for cheese manufacture,) make the situation

so critical that the manufacturer is left with no option but to dispose of it as waste, after possible treatment, regardless of the costs involved.

Burdock *et al.* (1967) reported the possibility of treating the whey with formalin for short storage periods at room conditions at the farm. However, no attempt at possible utilization of such chemically treated whey for yeast fermentation, has been reported.

The present investigation was initiated to study:

Part I -- The preservative effect of various chemicals on whey.

Part II -- The suitability of such chemically treated whey for the production of protein-rich yeast.

REVIEW OF LITERATURE

Whey is the greenish-yellow fluid remaining after the curd has been removed from the main product in cheese manufacture, from either whole or skim milk. The chemical composition of whey depends on the chemical composition of the milk from which it is made, the losses of milk constituents during cheese manufacture, and the usual practice to remove fat as whey cream (Whittier *et al.*, 1950).

WHEY COMPOSITION

Typical composition of whey as reported by Whittier *et al.* (1950) is as follows:

Water	93.0 %
Total solids	7.0 %
Lactose	4.9 %
Nitrogenous matter	0.9 %
Ash	0.6 %
Fat	0.3 %
Lactic acid	0.2 %
Riboflavin	1.24 ν /g

The differences observed in the composition of whey are attributable mainly to two factors, namely: (I) The method used in the coagulation of the casein, and (2) the extent to which fermentation of lactose has been allowed to progress (Whittier *et al.*, 1950).

The casein may be coagulated either by rennet, as in the case of soft cheese (e.g. Camembert cheese, etc.)

or it may be coagulated by means of acid added or produced in the milk by fermentation (e.g. cottage cheese), or by the combination of these two methods, such as is commonly employed in the manufacture of cheddar cheese (Thom et al., 1932).

When the coagulating agent is rennet, the calcium and phosphorus of the casein complex remain, for the most part, in the curd. However, the acid coagulation transfers phosphorus and most of the calcium to the whey, and thus the whey from rennet coagulated curd is less in ash than that of the whey from acid coagulation (Whittier et al., (1950). Hence, whey may be classified into types according to the particular method employed in the manufacture of cheese.

The major microorganisms present in whey come from the starter added during the manufacture of cheese, and which predominantly include species as Streptococcus lactis, and Streptococcus cremoris, or other bacteria for the production of volatile compounds of characteristic flavors (Van Slyke et al., 1963).

In addition to the microorganisms that come from the starter, the whey could contain the natural microflora of milk, depending on whether raw or pasteurized milk is used for cheese manufacture (Whittier et al., 1950). Also

it includes the microorganisms that are introduced during the manufacture of cheese, from rennet, color, equipment surfaces, and during handling, transportation, and storage of whey.

WHEY DISPOSAL AND UTILIZATION: A PROBLEM AND A POTENTIAL

According to an estimate by Webb et al., 1948, in the United States alone, about 10 billion pounds of whey are produced annually. And this volume of whey has a potential of about 500 million pounds of lactose, 50 million pounds of protein, 40 million pounds of non-proteineous matter, 11 million pounds of phosphorus, 7 million pounds of calcium and 12,000 pounds of riboflavin. However, overall figures may be much higher at present. In spite of such a high potential it was estimated (Forges, 1959; and Burdock et al., 1967/68), that in 1959, in the United States, only 29% of whey was utilized and the remaining more than 2/3 of the whole quantity, was discarded as a waste.

For disposal of whey it cannot be discharged into a river or larger system without pretreatment, because whey has an extremely high oxygen demand which can deplete the oxygen reserves of a waterway. Such pretreatment can be expensive, and so alternative methods are being sought

to utilize the whey as a raw material for various by-products.

The methods either employed or recommended for utilization of whey include returning the whey to farms for feeding, producing fuel gas by anaerobic fermentation, or further processing for ultimate utilization in feed, food or industrial products. However, due to the highly perishable nature of whey, its volume, the higher investment costs and lower reliability of certain processes (e.g. fuel gas by fermentation), the utilization of whey has not been considered very promising in the past (Whittier et al., 1950; Wasserman, 1960 a). As a result, the exploitation of whey potential has very rarely been carried out on a large scale, except under war-time emergencies. The disposal of whey as a waste involves high costs. In some parts of the United States the industry pays up to 3 cents/cwt. for hauling the whey from cheese plants. Further, the cost of treating whey in sewage treatment plants is very high because of its high biological and chemical oxygen demands (Porges, 1958, 1959). Therefore, it would be desirable to prolong the storage life of whey at room conditions at the factory or the farm, so that it could be conveniently and economically utilized, for the production of protein rich yeast etc.,

by fermentation, or as livestock feed.

PART I - PRESERVATION OF WHEY

Microflora, being abundantly present in the environment and associated with the raw material, are the major cause of short storage life, and of the deterioration of foodstuffs (Frazier, 1958). Therefore a large number of chemicals of organic and inorganic nature which are useful as anti-microbial agents have been suggested, and are in use for the preservation of food.

Among the organic chemicals, benzoic acid, sorbic acid, and lower fatty acids are commonly used. Various aspects of their effectiveness against bacterial, yeast and mold species, have been studied by many workers (Morse, 1951; Smith et al., 1952; and Desrosier, 1963).

Smith et al. (1952) observed that the germicidal activity of benzoic acid increased ten fold, in the food with pH 3.0 as against pH 7.0. Further, Desrosier (1963) reported that the germicidal action of benzoic acid was as much as 100 times more effective in highly acidic foods than in those with a slightly alkaline pH. He also found that benzoic acid was more effective against yeasts than molds and less effective against bacteria. He suggested that fatty acids are basically mold inhibitors and lack bactericidal capacity.

Desrosier (1963) in his review, reported that the chemicals of inorganic type rate highly effective as bactericidal agents. Those commonly in use in the food industry include sulfur dioxide, hydrogen peroxide, chlorine and carbon dioxide. Among these chemicals, carbon dioxide finds use in carbonated beverages and in physiological control of the storage quality of fresh fruits, rather than as a chemical preservative. On the other hand, chlorine finds wide use as a chemical disinfectant, and in the treatment of water for drinking and food processing, rather than as a chemical preservative (Desrosier, 1963). The most effective action of chlorine has been observed at low pH values (LaBree et al., 1960).

However, among all the chemicals, only sulfur dioxide and hydrogen peroxide appear to be promising since the organic chemicals are not suitable for the proposed investigations, for reasons pointed out in the preceding discussion, and which are summarized below:

(i) the organic chemicals have comparatively low inhibitory effects against bacterial species, whereas the chemical treatment desired must be effective against whey microorganisms which mainly comprise bacterial species of milk origin,

(ii) due to relatively high costs, the use of organic chemicals does not seem feasible for treatment of the whey, and

(iii) organic chemicals have relatively high inhibitory effects against yeast species, and such chemical treatment could then render the whey unsuitable for yeast propagation.

(i) Sulfur Dioxide

Sulfur dioxide is used as a chemical preservative to reduce or prevent spoilage by microorganisms in fermentation industries (Desrosier, 1963). It is used in its gaseous or liquid form, or dissolved in water to form sulfurous acid or in the form of its natural or acid salts (Joslyn et al., 1954).

Sulfur dioxide has been used in the preservation of foods for centuries (Desrosier, 1963). Bioletti (1911) reported that the use of fumes of burning sulfur in wine making was known to early Egyptians and Romans. Joslyn et al.(1954) reported that sulfur dioxide was used for the preservation of meats in the United States as early as 1813. They also reported that in Europe initially the use of sulfurous acid was introduced about the beginning of the Nineteenth century, originally for purification in the

beet sugar industry rather than as a chemical preservative.

Due to its relatively low cost, convenience in use, and the ease with which its concentration can be reduced to a level within tolerable limits, sulfur dioxide finds wide use for a variety of food products (Joslyn et al., 1954; Desrosier, 1963).

The form and the particular source of sulfur dioxide employed for food preservation depends on the nature of the food product and the available sulfur dioxide content of the source (Joslyn et al., 1954). An account of various preparations and their theoretical and actual available sulfur dioxide contents, is given in Table 1.

(ii) Effect of pH on the Preservative Action of Sulfur Dioxide

In water, sulfur dioxide exists as the dissolved gas, as undissociated sulfurous acid(H_2SO_3), as the bisulfite ion (HSO_3^-) and as the sulfite ion (SO_3^{--}) (Joslyn et al., 1954). The germicidal action of sulfur dioxide is considered to be due to sulfurous acid in its undissociated form and not due to bisulfite ions (Muller et al., 1914; Cruess et al., 1931, 1932; Cruess, 1932). Ingram (1949) demonstrated that at pH 9.5 and above, only SO_3^{--} ions exist. The pH ranging from 9.5 - 4.5 is

TABLE 1. Available sulfur dioxide content of various sources

Compound	Formula	Available percent of SO_2	
		Theoretical	Actual
Liquid sulfur dioxide	SO_2	100.0	100.0
Acid sulfurous, 6%	H_2SO_3	6.0	6.4-6.8
Calcium sulfite	$Ca\ SO_3 \cdot 1\frac{1}{2}\ H_2O$	23.0	43-45
Potassium sulfite	K_2SO_3	33.0	36
Sodium sulfite	Na_2SO_3	50.84	48
Potassium bisulfite	$K\ H\ SO_3$	53.31	-
Sodium bisulfite	$Na\ H\ SO_3$	61.59	55
Potassium metabisulfite	$K_2\ S_2\ O_5$	67.43	52
Sodium metabisulfite	$Na_2\ S_2\ O_5$	57.65	61

* Joslyn *et al.* (1954).

suitable for the existence of both the HSO_3^- and SO_3^{--} ions. Further, he suggested that though HSO_3^- ions could occur in pH values ranging from 8.0 - 0.0, whereas H_2SO_3 could exist only at pH 4.0 and below. Thus, even slight change in pH in the region of 3.5 and above, would markedly affect the proportion of undissociated sulfurous acid and reduce the germicidal or inhibitory effect of sulfur dioxide.

(iii) Hydrogen Peroxide

The bactericidal property of hydrogen peroxide has been recognized for a long time (Nambudripad, 1949). On account of its relatively high bactericidal power and the ease of removal of residues, among all the preservatives in use, H_2O_2 has been found to offer the greatest possibilities for increasing the keeping quality of milk (Budde, 1903; Satta, 1943; Giolitti, 1951 a; Pien 1951; and Nambudripad, 1949).

One of the earliest attempt in this field consisted of treating the milk with H_2O_2 (final conc. 0.03 - 0.036%) and holding it at 50°C for 2 hours (Budde, 1903). The milk so treated was claimed to remain in good condition for several days. Further, the use of electrolytically purified hydrogen peroxide was suggested, so as to eliminate

the impurities of arsenic etc., and the process (Satta, 1943) was adopted during the Second World War in Italy and other European countries for the treatment of milk for transportation to distant places (Nambudripad, 1949).

Morris et al. (1951) showed that treatment of milk with 0.2% hydrogen peroxide followed by addition of catalase, compared well with pasteurization in all respects. Menae (1952) reported that the milk samples treated with immediate addition of 0.4, 0.6 and 0.8 per cent (130 vol.) hydrogen peroxide, had better keeping quality than pasteurized ones, when stored at 13°C.

Giolitti (1952) indicated that the milk samples treated with 0.1% (130 vol.) H_2O_2, showed no significant change in composition.

(iv) Time of Exposure

Giolitti et al. (1952) found that all the susceptible organisms were destroyed after an exposure of 8 hours, when milk samples with considerably higher initial bacterial counts were treated with 0.8% (130 vol.) H_2O_2.

Nambudripad et al. (1949,1951) in a series of experiments studied the effects of exposure to H_2O_2 on various bacterial and yeast species isolated from milk. He observed that the time of exposure was greatly reduced when the H_2O_2 concentration was increased from 50 to 300 p.p.m., and

it required 3/4 - 7 hours for complete destruction of common milk spoilage bacteria at 300 p.p.m. H_2O_2 concentration. The spores of aerobic, mesophilic spore-forming bacteria were found least susceptible, and complete destruction at 50 p.p.m. and 300 p.p.m. occurred in 24 - 120 hours and 14 - 32 hours respectively. The yeast species were destroyed completely in 24 - 26 hours and 7 - 8 hours at 50 p.p.m. and 300 p.p.m. H_2O_2 concentration, respectively.

With regard to the effectiveness against pathogenic organisms, Ciolitti (1951 b) found that Escherichia coli and Brucella abortus were destroyed by 72 hours exposure to 0.6% H_2O_2 (130 vol.).

(v) Effect of pH on the Preservative Action of Hydrogen Peroxide

As indicated earlier, the efficiency of preservation of foods by benzoic acid is reported to increase greatly, by lowering the pH of the medium (Smith et al., 1962). However, no attempts have been made to study the effects of pH on the efficiency of preservation by hydrogen peroxide. Since it is intended to use chemically preserved whey for yeast propagation, the lowest H_2O_2 concentration and maximum possible efficiency of preservation are desired.

(vi) Mechanism of Microbial Inhibition

Microflora are essentially masses of cell protoplasm containing enzymes so organized as to perform the orderly progress of metabolic processes through the following factors: (1) a genetic mechanism regulating reproduction, (2) a living membrane separating the cell contents from direct contact with the environment, and (3) enzymes and enzyme systems vital for overall cell metabolism (Wyss, 1948). Severe disturbances of any of these factors result in the death of the cell, and trivial disturbances of any of these factors result in partial or complete inhibition of cellular activity. Disruptive influences may vary from profound effects such as the actual disruption of cells by physical forces e.g. heat, irradiation and supersonic waves, to the slowing or complete inhibition of metabolic activities by chemical agents (Wyss, 1948). A vast quantity of literature has been accumulated on each of the above subjects. This review will deal only briefly with the mechanism of antimicrobial action brought about by chemical substances within the scope of this investigation.

The chemicals used as preservatives inhibit or kill the undesirable microorganisms in food products, by interfering with either one or more of their genetic

mechanisms, cell membranes, and enzyme systems (Wyss, 1948).

As reviewed by Wyss (1948) a widely adopted approach has been to interfere with the genetic mechanisms to inhibit or kill (a bacterium is considered dead when it has lost power to reproduce - Wyss, 1948) the micro-organisms by denaturation of its genetic substances, or retarding its growth by inducing mutations. He reported that in a number of bacterial species and fungi, it was possible by ultraviolet and X-ray radiations to induce mutations involving the loss of ability to synthesize a vitamin or amino acid.

The chemicals employed to kill or inhibit microbial growth by interference with the cell membrane, could do so either because of their ability to diffuse into the cell and unite with the cellular components, e.g. chlorine (Chang, 1944), or by damaging the cell membrane e.g. the fat solvents, or by the action on cell membrane as a surface active agent (Hotchkiss, 1946).

The majority of the chemical preservatives used in the food industry e.g. sulfur dioxide plus its salts, hydrogen peroxide, benzoic acid, and sorbic acid etc. fall in the category of chemicals that result in microbial inhibition or death by interference with the enzymatic activities of the cell (Wyss, 1948). Increasing acidity

through its higher hydrogen ion concentration, is another major factor contributing to the inhibition of microbial growth through irreversible denaturation of the colloidal nature of cellular enzymes, at large departures in pH from the optimum for enzyme action (Wyss, 1948; Smith et al., 1962).

(vii) Microbial Inhibition by Sulfur Dioxide

The preservative effect of sulfur dioxide in fruit and vegetable products and other foods and beverages was discussed by Abdulov, 1938; Cruess, 1948; Borgstrom,1953; Tanner, 1944; von Schelhorn, 1951; and Woodroof et al., 1945. The actual mechanism of preservative action of sulfurous acid and its salts, however, is not known (Wyss, 1948). It is believed that its strong reducing power may be involved in two ways in inhibiting and germicidal action, namely: (1) by reducing the oxygen tension in the food tissues and beverages to a point below which aerobic microorganisms cannot grow, and (2) by inactivating the disulfide enzymes of cellular system by splitting the disulfide (S-S) linkage which is essential for enzyme activity, into -SH groups (Wyss, 1948).

(viii) Microbial Inhibition by Hydrogen Peroxide

Evidence from the work of Hellerman (1937) and the studies of the enzymes tabulated by Singer (1946) indicate that the active sulfhydryl (-SH) group is essential for the activity of respiratory enzymes and hydrolases. Hellerman (1937) found that oxidizing agents such as hydrogen peroxide and halogens etc. were able to destroy the cellular activity of these enzymes by oxidizing the active sulfhydryl groups into inactive disulfide group in a manner indicated below:

$$\underset{\text{(active)}}{2\ \text{Enzyme} - \text{SH}} \longrightarrow \underset{\text{(inactive)}}{\text{Enzyme-S-S-Enzyme}} + 2\ \text{H}$$

PART II - YEAST PROPAGATION

As was mentioned earlier, a multidirectional approach has been made to economically utilize or dispose of food industry waste. Most of these are too dilute for economic recovery of solids by chemical or other means. However, yeast propagation could offer economic utilization because of its ability to utilize organic solids from a relatively dilute medium efficiently, when supplemented with inorganic nutrients (Wasserman, 1960 a, Graham et al., 1953 a,b,c).

In whey, lactose is the chief source of available carbohydrates (Whittier et al., 1950). Growth of any microorganism in whey depends on its ability to utilize lactose. The species of yeasts capable of assimilating lactose are very limited (Wasserman, 1960).

Hogosa et al. (1947) found that Torula cremoris was satisfactory in utilizing whey lactose for production of ethyl alcohol. Further, Torula cremoris was also found most promising for yeast production among four test species, namely: (i) Candida krusei, (ii) Torula utilis, (iii) Torula utilis thermophilus, and (iv) Torula cremoris (Graham et al., 1952).

Porges et al. (1950, 1951) and Wasserman (1960) in a series of investigations, observed that Saccharomyces fragilis grew more rapidly than several other species of yeasts. Accordingly, in the present investigations Torula cremoris and Saccharomyces fragilis were chosen as the test organisms for rapid conversion of fresh and chemically treated whey into yeast protein.

(i) Effect of Temperature

Graham et al. (1952) reported that the best growth of Torula cremoris was obtained at 26°C. Wasserman (1960) found that good yields were obtained at temperatures as high as 41-43°C. However, he observed that at such high

temperatures, bacterial contamination occurred. The optimum temperature for the growth of Saccharomyces fragilis was 31-33°C.

(ii) Effect of pH

Graham et al. (1952) reported the optimum pH for the growth of Torula cremoris was 4.4; however, good yields were also obtained even at a pH range as high as 5.9 - 6.8. Wasserman (1960) reported that the maximum growth of Saccharomyces fragilis occured at pH 5.0 - 5.7.

(iii) Rate of Aeration

Wasserman et al. (1961) found high yields were dependent on the supply of sufficient oxygen to satisfy the oxygen demand of yeast. The peak oxygen demand, which is described as the minimal quantity of oxygen that must be supplied to microorganisms at the time of greatest activity, was found for yeast to be 4.75 millimoles of oxygen per liter of medium per minute ($mMO_2/l/min.$).

However, the oxygen absorption rate (O.A.R.) which signifies the extent of oxygen being absorped by the fermentation medium, largely depends on the design of propagation equipment in respect to its air dispensing, impelling, and foam breaking devices (Webb, 1964).

(iv) Inorganic Supplementation

Although whey contains nitrogen, phosphorus, and other nutrients (Whittier et al., 1950); Wasserman (1960 a) found that supplementation was necessary for maximum yeast yields. He reported that the ratio of carbon to available nitrogen in whey is 48:1 approximately, whereas in yeast the average carbon to nitrogen ratio is 7:1. Further, since the whey contains approximately 0.14% nitrogen of which only about 25% is actually available to yeast, the addition of inorganic nitrogen is necessary in order to correct the deficiency, and to meet the required C:N ratio to support the growth of yeast. He further suggested that the supplementation of whey medium with approximately 0.85% ammonium sulfate and 0.225% phosphorus as phosphate, was sufficient to correct the deficiency.

(v) Lactose Concentration

The lactose content of whey ranges from 4.0 - 5.0%, depending on the variety of cheese produced (Whittier, et al., 1950). Graham et al. (1953 b) studied the effect of initial lactose concentration of whey on the growth of Torula cremoris. He reported that when whey was diluted to a lower concentration or concentrated to nearly double

the lactose content, the yeast growth and overall efficiency in lactose utilization was relatively poor. In normal whey (i.e. with no dilution or concentration), optimum growth and efficiency of lactose utilization was observed.

(vi) Heat Treatment

Heat treatment of whey has been recommended for higher yeast yields by Hanson et al. (1949), and Whittier et al. (1950). Enebo et al. (1941, 1942) found that the growth of Saccharomyces fragilis was increased by 50% in heat treated and filtered whey. But Wasserman et al. (1959) and Wasserman (1960) reported that the growth of Saccharomyces fragilis in raw whey medium was similar to that of heat-treated deproteinized whey. However, since the above observations are conflicting, this aspect needed further investigation.

SCOPE OF INVESTIGATION

The scope of this investigation was to study the preservative effect of various chemicals on whey; and the suitability of such chemically preserved whey for production of protein rich yeast. Specifically the following areas were investigated:

(i) The optimum conditions for preservation of whey by sodium sulfite, sodium metabisulfite, and hydrogen peroxide,

(ii) The utilization of chemically preserved whey by *Torula cremoris*, and *Saccharomyces fragilis*.

MATERIALS AND METHODS

Whey, from the manufacture of cheddar cheese from pasteurized milk, was obtained from the commercial Dairy, University of Manitoba.

Experiment I was designed to study the effectiveness of sodium sulfite, sodium metabisulfite, and hydrogen peroxide for preservation of whey with respect to the concentration of chemical used, and the pH of the whey medium.

The criteria considered for effectiveness of chemical treatment were:

(i) the reduction in the viable bacterial cell count, originally present and,

(ii) the extent of decline in the original lactose content of chemically treated and non-treated whey, during storage at 70 - 72°F.

In Experiment II, the growth pattern of yeast species namely Torula cremoris, and Saccharomyces fragilis in natural whey medium, and the suitability of chemically treated whey for yeast propagation were studied.

Utilization of lactose and the corresponding growth of yeast in terms of cell count and centrifuged solids were considered as measures of efficiency.

EXPERIMENT I: PRESERVATION OF WHEY

(i) Source of Chemicals and Enzyme

Sodium sulfite ($Na_2SO_3.7H_2O$), Analar, British Drug House, England.

Sodium metabisulfite ($Na_2S_2O_5$), Analar, British Drug House, England.

Hydrogen peroxide (H_2O_2, 35%), C.I.L., Montreal, Canada.

Catalase (a powder preparation from beef liver), Sigma Chemical Co., St.Louis, Missouri, U.S.A.)

(ii) Variables

Initial phase - (i.e. preliminary investigations)

(a) Control

0.0 (no chemical) at pH 5.25

(b) Sodium sulfite

0.04, 0.10, 0.16, and 0.20% at pH 5.25.

(c) Sodium metabisulfite

0.01, 0.02, 0.04, and 0.10% at pH 5.25.

(d) Hydrogen peroxide

50, 125, 250, and 500 p.p.m. at pH 5.25.

Final phase -

(a) Control

0.0 (no chemical) at pH 3.5, 4.4, 5.0, 6.0 and 7.0.

(b) Sodium sulfite

(i) 0.10, 0.20, 0.30, and 0.40% at pH 5.0.

(ii) 0.40% at pH 3.5, 4.4, 5.0, 6.0, and 7.0.

(c) Sodium metabisulfite

(i) 0.05, 0.10, 0.20, and 0.30% at pH 5.0.

(ii) 0.15% at pH 3.5, 4.4, 5.0, 6.0 and 7.0.

(d) Hydrogen peroxide

250 p.p.m. at pH 3.5, 4.4, 5.0, 6.0, and 7.0.

(iii) Experimental Procedure

Erlenmeyer flasks of 250 ml capacity were sterilized at 121°C for 15 minutes, then 200 ml of fresh whey was transferred into each flask and the desired pH was obtained by addition of 0.1 N HCl or 0.1 N NaOH. The respective quantities of chemicals were added, and the flasks were identified accordingly. Treated samples were stored at room temperature (70-72°F). Samples in aliquots of 30 ml were drawn before and also after 3, 10, and 21 days of storage. Aseptic conditions were maintained throughout the investigation, when necessary.

The samples were examined for changes in pH, viable bacterial cell count, and the lactose content, as described under laboratory methods.

EXPERIMENT II: YEAST PROPAGATION

(i) Test Organisms

(i) Torula cremoris Y - 14. (obtained from Department of Dairy Science, University of Saskatchewan.

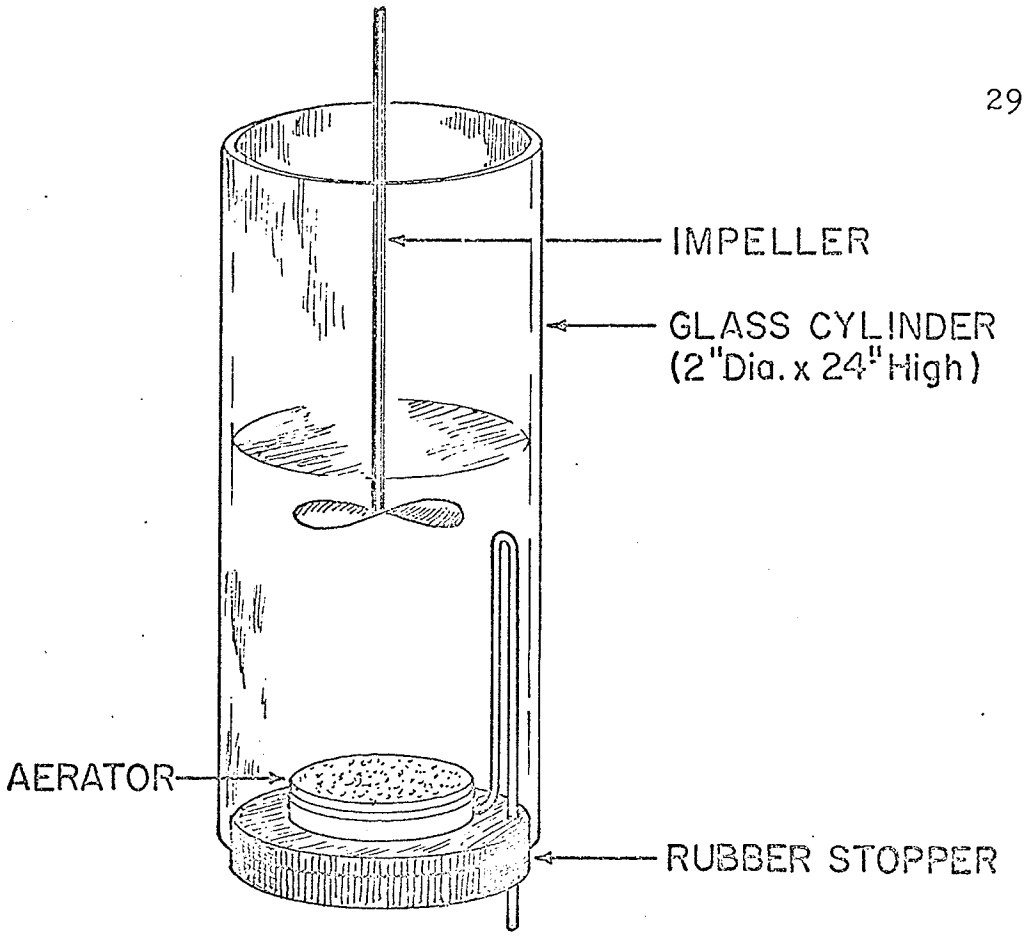
(ii) Saccharomyces fragiles Y - 1109. (obtained from A.T.C.C., Rockville, Maryland, U.S.A.)

(ii) Fermenter Design

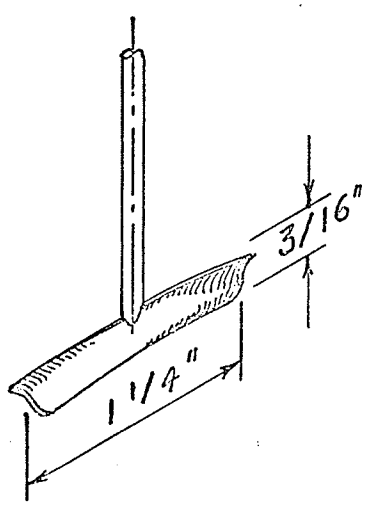
Propagation studies were carried out on shaker and in two laboratory fermenters designed for the purpose.

Fermenter Model-I was constructed of a glass cylinder of 2 inches diameter and 2 feet length, with open ends. One end of the cylinder was closed by a rubber stopper to act as the bottom of the fermenter. The air dispersing device consisted of a fine U shaped glass tube with an air dispersing glass disc of 1 1/8 inches diameter, fitted at the bottom, with its other end connected to an air supply (Figure 1).

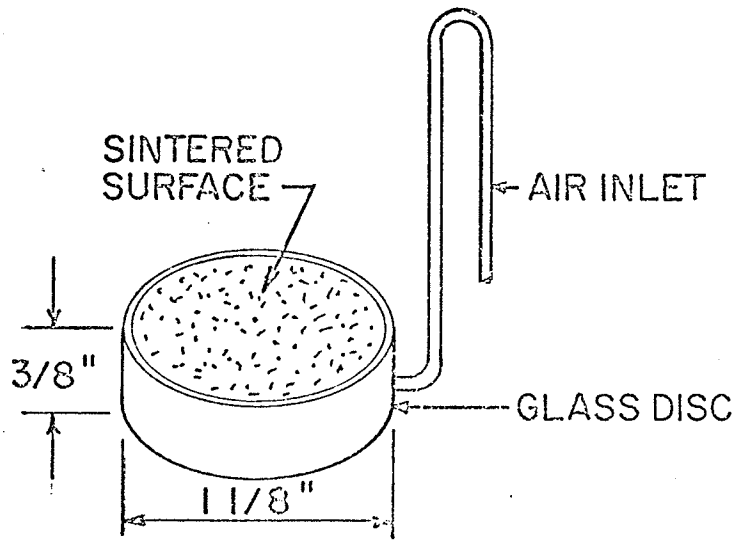
Fermenter Model - II was constructed of a 10 1/8"x18" glass jar, with a volumetric capacity of 4 3/4 gallons. For dispersion of air into the fermentation medium, a stainless steel bowl of 6½" diameter and 1½" depth was used. Compressed air was passed into the bowl through a side inlet of 3/16" diameter, and the air was dispersed



FERMENTER ASSEMBLY (n.t.s.)



STAINLESS STEEL IMPELLER



AERATOR

Fig. 1 FERMENTER MODEL I (capacity about 600 ml)

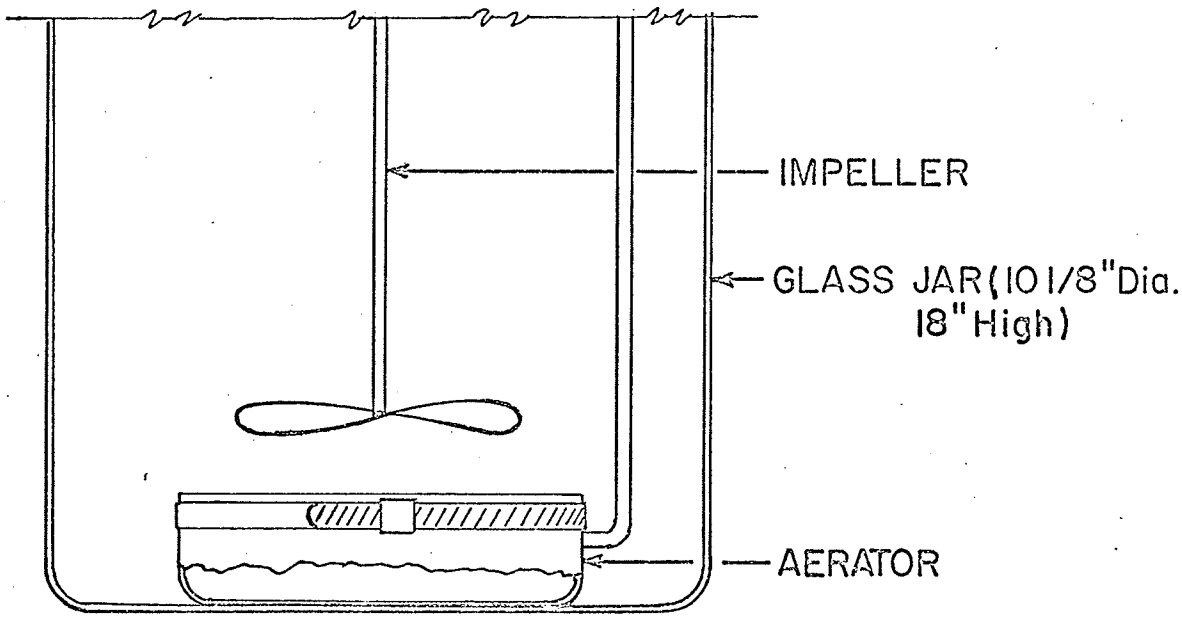
into the medium in the form of fine bubbles through a double layer of filter cloth, which was tightened on the top of bowl by screw clamps. In addition the bubbles were stirred by an impeller driven by a 1/12 H.P. motor, as to facilitate absorption of oxygen into the medium (Figure 2).

(iii) Inoculum

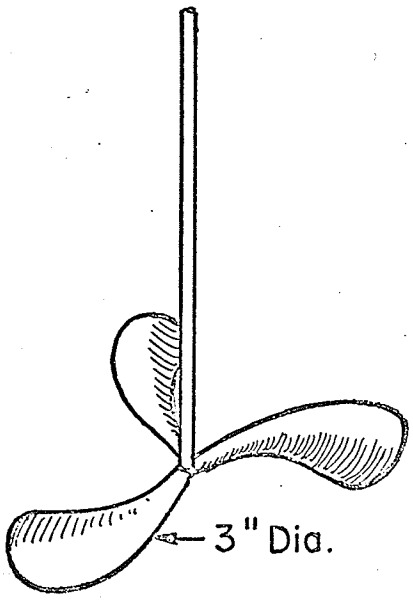
Each inoculum was prepared in two ways, hereafter referred as Type A and Type B.

Type A - The whey was supplemented with 0.25% urea plus 0.30% diammonium phosphate and the pH of the whey was adjusted to the optimum for the growth of each test organism (i.e. 4.4 for Torula cremoris and 5.0 for Saccharomyces fragilis, as per details under Results). After adjusting the pH, the whey in 50-75 ml volumes was placed in 250 ml Erlenmeyer flasks, and sterilized at 121°C for 15 minutes. When cooled, the flasks were inoculated with stock culture of the test organism and incubated in a shaker - incubator for 24-48 hours at 26-28°C for Torula cremoris and at 32°C for Saccharomyces fragilis.

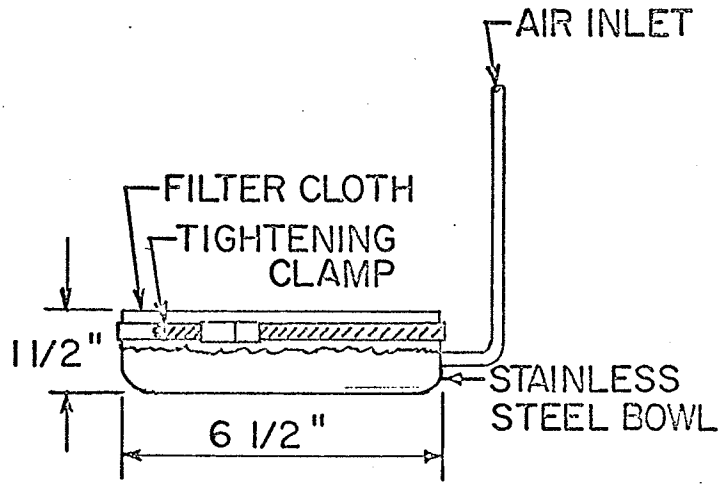
Type B - A nutrient-rich broth (hereafter called inoculum broth) of the composition given in Table 2



FERMENTER ASSEMBLY (n.t.s)



STAINLESS STEEL
IMPELLER



AERATOR

Fig. 2 FERMENTER MODEL 2 (capacity about 6 liters)

TABLE 2. Composition of inoculum broth used for preparation of inoculum Type B.

Lactose	2.50%
Yeast extract	0.50%
Potassium phosphate	0.50%
Diammonium phosphate	0.25%
Bacto peptone	0.50%
Beef extract	0.30%

(and details in Appendix 1), was used as the culture medium. Erlenmeyer flasks of 250 ml containing 50-75 ml of inoculum broth were sterilized at 121°C for 15 minutes, cooled, and inoculated with the test organism from slant cultures. Then the inoculated flasks were incubated for 24-48 hours in a shaker-incubator at 26-28°C for Torula cremoris and at 32°C for Saccharomyces fragilis. The culture obtained by this method was called stock culture. The Model-II fermenter (fermenting capacity about 6 liters) was sterilized in autoclave at 121°C for 15 minutes, and 4 liters of sterilized inoculum broth were put into it. Further, 800 ml of stock culture was added into the fermenter (this gave an inoculum broth to stock culture ratio of 5:1) and the propagation was carried out for 12-16 hours. During propagation of Torula cremoris, the temperature was maintained at 26-28°C and the pH at 4.4. In case of Saccharomyces fragilis, the temperature was maintained at 32°C and the pH at 5.0-5.5. At the end of 12-16 hours of fermentation, a yield of about $1,200 \times 10^6$ yeast cells per ml of culture was obtained. This culture was used as an inoculum and referred to as Type B. The inoculum prepared by this procedure was found to give a relatively vigorously growing culture, and also higher cell count per ml of inoculum, as compared to inoculum Type A, described earlier.

(iv) Heat Treatment

In order to study the effect of the heat treatment on whey utilization by yeast species, heat-treated and raw whey was used for yeast propagation. The heat treatment was carried out at 104°C for 15 minutes, and at 70°C for 5 minutes.

(v) Temperature

The desired temperature (i.e. 26-30°C for Torula oremoris and 32°C for Saccharomyces fragilis as indicated under Results) was maintained during fermentation by installing the fermenters in a water bath. The temperature of the water bath was controlled by a heating coil and a thermostat connected to it (Figure 3).

(vi) Air

Compressed air was filtered by passing through an activated carbon filter. The level of air flow supplied to the fermentation medium was regulated with the aid of an air flow meter. The oxygen absorption rate (O.A.R.) as $mMO_2/l/minute$ was determined by the sulfite oxidation method (Webb, 1964; Cooper et al., 1944; and Wasserman et al., 1961).

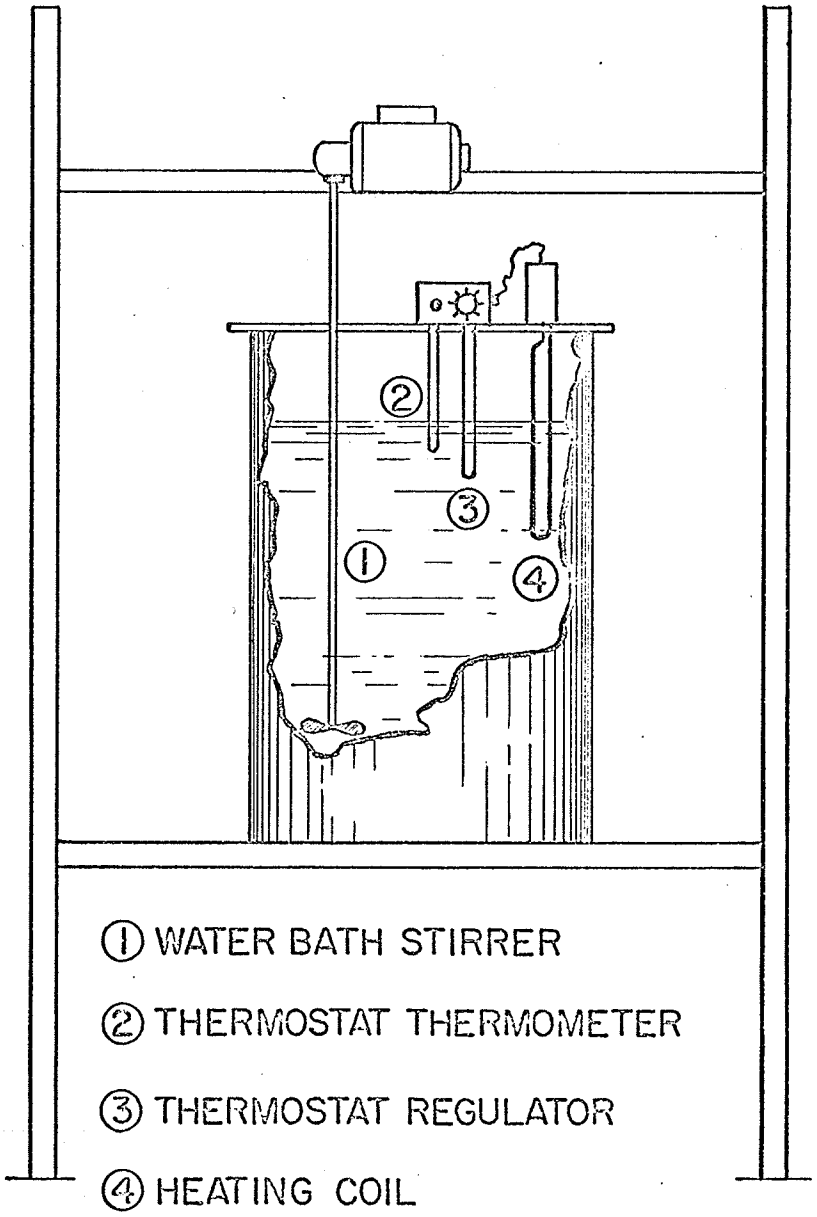


Fig. 3 WATER BATH AND THE FERMENTER OUTFIT

(vii) pH

In the fermentation studies, pH 4.4-5.0 was employed for Torula eremoris, and pH 5.0-5.7 was employed for Saccharomyces fragilis. The initial pH of the fermentation medium was adjusted to the desired level and the change in the pH during fermentation was monitored at intervals of about 30 minutes during the first 3 hours, and at intervals of about 2 hours at the later stages. The changes in the pH during the first 2-3 hours were rapid and only slight changes occurred during later fermentation. The changes in pH were corrected by the use of 0.1 N acetic acid and 0.1 N NaOH, when necessary.

(viii) Foam Control

Excessive foaming during fermentation was controlled by using a small quantity of 10% Dow Corning Antifoam-C emulsion.

LABORATORY METHODS

(1) Chemical Analysis

Determination of total solids -

A 2.5-3.0 gm sample was weighed into a flat bottom dish of not less than 5 cm in diameter. The sample was

heated on a steam bath for 10-15 minutes or until it evaporated to dryness. Then it was heated in an air oven for 3 hours at 98-100°C, cooled in a desiccator and weighed quickly. The percentage of total solids was calculated from the weight of the residue (A.O.A.C.,1965).

Determination of lactose content -

The lactose content was determined by a modified Fehling's technique. A 25 ml sample was mixed with 25 ml of 10% neutral lead acetate, made to volume in a 100 ml volumetric flask and filtered. A 50 ml aliquot was further added with 12.5 ml of 10% potassium oxalate, made to volume in a 100 ml volumetric flask and filtered. The aliquot so obtained was used to titrate 5 ml of Fehling's A solution plus 5 ml of Fehling's B solution, while boiling, and using methylene blue as indicator (Lane, and Eynon, 1940). The lactose in the samples was determined by comparing the titration values with that of a freshly prepared 0.2-0.4% standard lactose solution. The dilution of samples was adjusted so that the titration value ranged between 15-35 ml. (Figure 4).

Determination of acidity-

To determine the acidity of whey, a 20 gm sample was weighed into a suitable dish, diluted with twice its volume

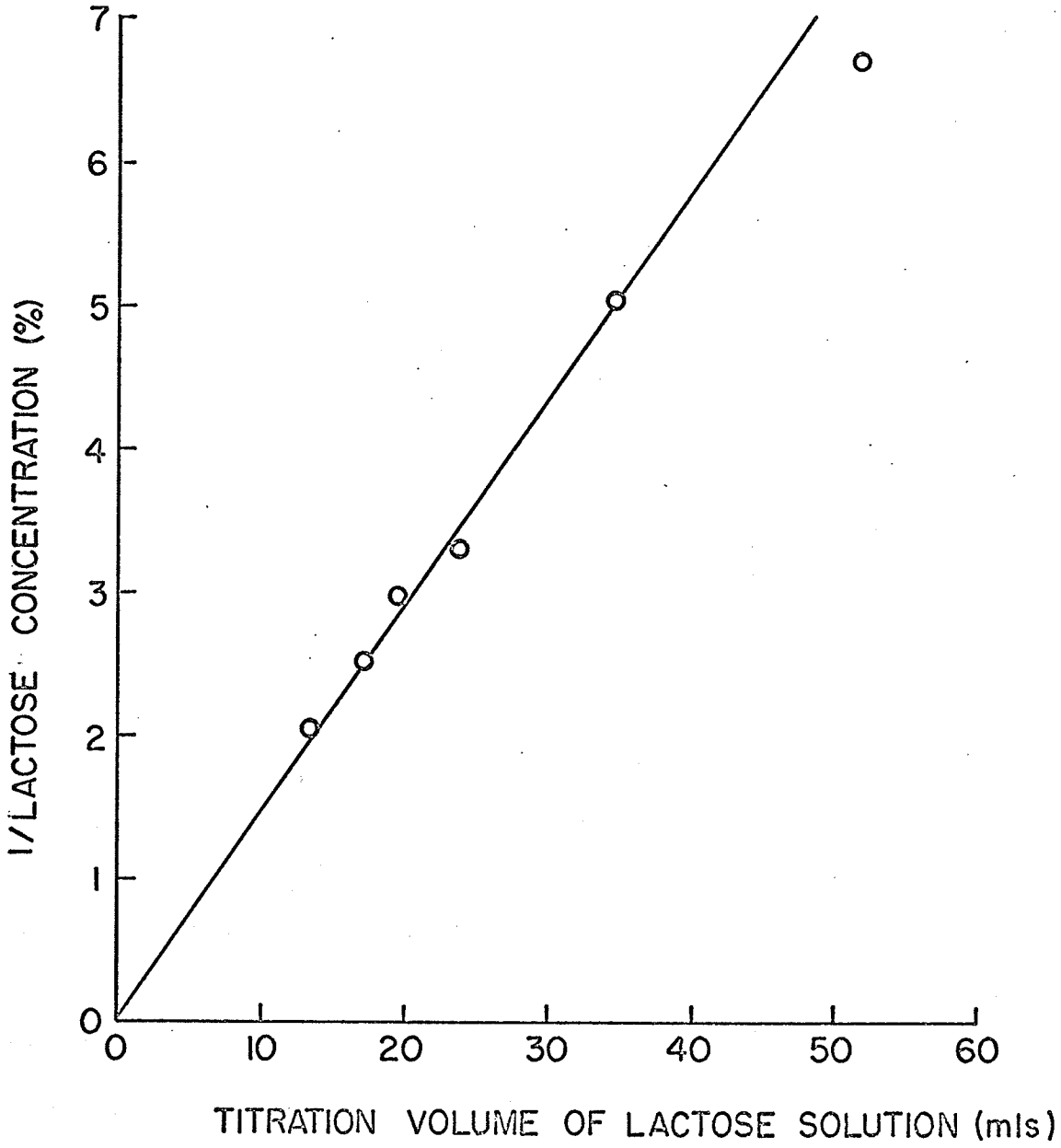


Fig.4 The Standard for Lactose Determination by Lane-Eynon's Method

[1/ Lactose Conc.(%) vs. Titration Volume of Lactose Solution]

of water and titrated with 0.1 N NaOH using 3 drops of phenolphthalein as indicator. The acidity was calculated and reported as a percentage of lactic acid.

Determination of pH -

The pH of whey was determined using a dual electrode Corning pH meter.

Determination of chemical oxygen demand (C.O.D.) -

The chemical oxygen demand of whey samples was determined by a modified A.P.H.A. (1965) method. The procedure consisted of the oxidation of 20 ml (diluted) sample with 10 ml of 0.25 N potassium dichromate in the presence of 30 ml of H_2SO_4-$AgSO_4$ reagent, and 0.4 gm of $HgSO_4$ as a catalyst. The oxidation was carried out by incubation for 10 minutes at 92°C in water bath. The oxidized sample was titrated against ferrous ammonium sulfate using ferroin as indicator.

Determination of centrifuged solids -

The centrifuged solids were determined by centrifuging the fermented medium at about 7,000 rpm (6,000 x g) for 15 minutes. A "Sorvall" Superspeed centrifuge Model RC-2B with rotor Head No.SS-34 (r=10.8 cm) was employed. The centrifuged solids were transferred into a porcelain dish,

and evaporated to dryness on a steam bath. The sample was then heated for 3 hours at 98-100°C, cooled in a desiccator and weighed. The residue was expressed as % centrifuged solids.

(ii) Bacteriological Analysis

Bacterial cell count -

The number of viable bacterial cells in whey was determined by the method recommended by A.P.H.A. (1965). Using a serial dilution plating technique, the sample was plated in duplicate on standard plate count agar. The plates were incubated at 32°C for 48 hours. The average of colony counts of a suitable dilution containing 30-300 colonies was selected to enumerate the viable bacterial cells per ml of whey.

Yeast count -

The yeast count of fermented whey was determined by direct microscopic examination using a hemacytometer as employed by Wasserman et al., 1961.

RESULTS

PART I: PRESERVATION OF WHEY

The average chemical composition of a typical sample of whey used in this study is given below (Appendix 2):

Moisture	93.25%
Total solids	6.75%
Lactose	4.65%
Nitrogenous matter	0.93%
Ash	0.58%
Fat	0.35%
Acidity	0.15%

Although there was not much variation in chemical composition of the whey from one lot to another, the total solids content ranged from 6-9%. The lactose content was the major constituent of the whey and the quantity ranged from 4.5-5.5%. The average content of the nitrogenous matter, ash and fat content of the whey were 1%, 0.6% and 0.35% respectively. The acidity of whey was found to vary from 0.13-0.15%, and the pH values ranged from 5.0-5.5. The chemical oxygen demand (C.O.D.) which is dependent mainly on the organic matter, varied between 52,000-55,000 mg/l. The original microbial population in the whey varied between 10,000 to 200,000 per ml.

(i) Preliminary Investigation

As there was no information available in the literature on the efficiency of chemical preservation of cheddar cheese whey, a preliminary study was aimed to ascertain the effectiveness of different chemicals in the preservation of whey. The effects of varying concentrations of chemical preservatives in whey (pH 5.25) stored for 7 days at room temperature, are shown in Table 3.

The data revealed that sodium sulfite at 0.1% was able to prevent substantial microbial growth in the whey. However, at levels of 0.16% and 0.2%, sodium sulfite was effective in inhibiting the bacterial population of the whey (Table 3, Figure 5). Sodium metabisulfite appeared to be more effective than sodium sulfite, as 0.1% of sodium metabisulfite not only could prevent growth but also reduced the original cell count of 9.4×10^3 per ml to 42 per ml. Of the three chemical preservatives, hydrogen peroxide was found to be most effective. Hydrogen peroxide at a level of 125 p.p.m. was able to arrest microbial multiplication, and at levels of 250 to 500 p.p.m. of hydrogen peroxide it was able to reduce the bacterial count to zero.

TABLE 3. The effect of chemical concentrations on the viable bacterial cells of the cheddar cheese whey, after 7 days of storage at room conditions (pH 5.25).

Chemical preservative		Concentration	Viable bacterial cells per ml. (Average of 2 trials) Initial	After 7 days
(1)	Control	0.00	9.4×10^3	6.2×10^6
(ii)	Sodium sulfite	0.04%	"	13.1×10^5
	"	0.10%	"	19.8×10^3
	"	0.16%	"	3.5×10^3
	"	0.20%	"	3.9×10^2
(iii)	Sodium metabisulfite	0.01%	"	1.5×10^6
	"	0.02%	"	12.5×10^5
	"	0.04%	"	25.2×10^4
	"	0.10%	"	4.2×10^1
(iv)	Hydrogen peroxide	50 ppm	"	12.4×10^5
	"	125 "	"	5.5×10^3
	"	250 "	"	0.0
	"	500 "	"	0.0

Appendix Tables: 3,4.

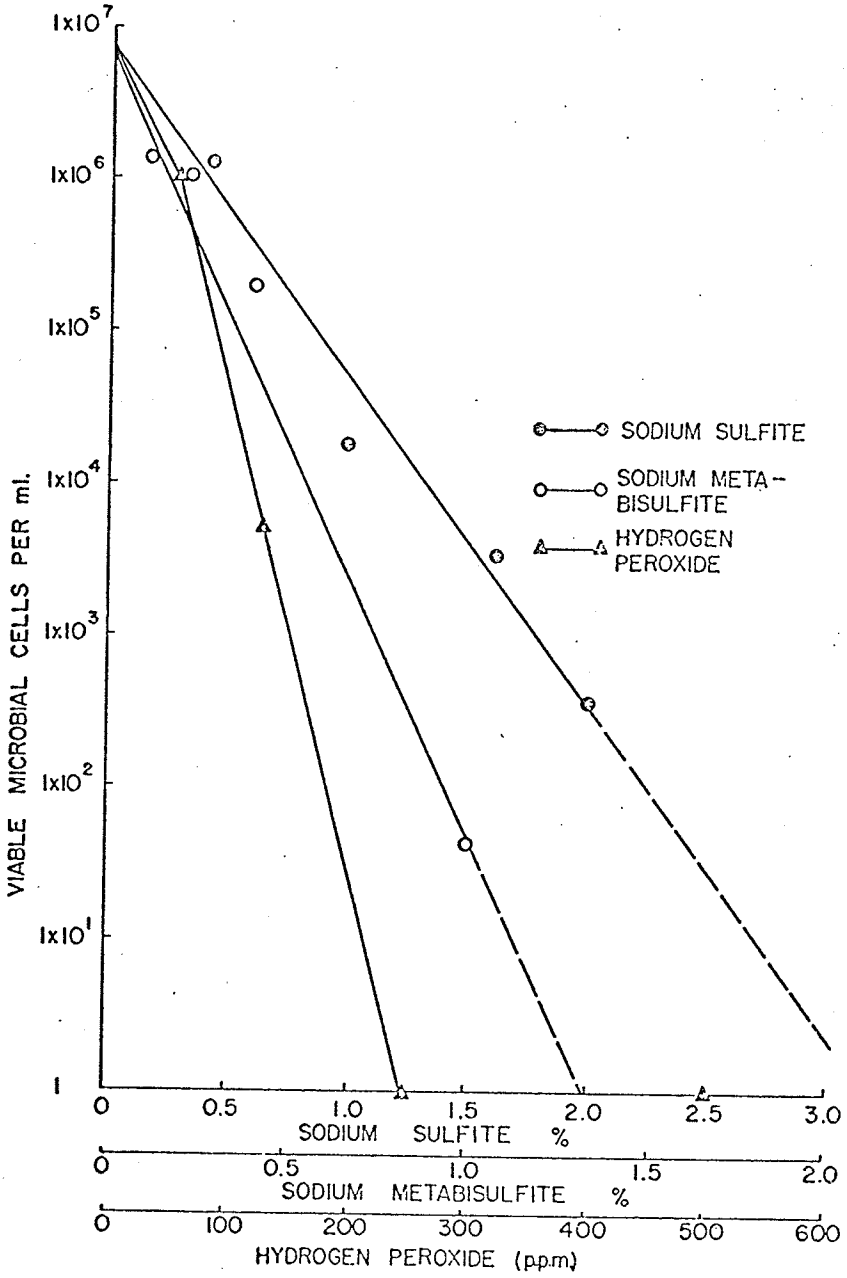


Fig.5 The Effect of Chemical Concentration on Whey Microorganisms During 7 Days of Storage at 70-72° F

(ii) The Effectiveness of Sodium Sulfite on the Preservation of the Whey

The effectiveness of the whey preservation was measured by performing the viable bacterial count of the whey and the lactose content of the whey. As preliminary data (Table 3) revealed that a level of 0.16% sodium sulfite was effective in lowering the initial cell population of the whey, various concentration of sodium sulfite ranging from 0-0.4% were added to the whey in order to determine its effectiveness as a whey preservative at room temperature. The results presented in Table 4 showed that at levels of 0.3% and 0.4% sodium sulfite, the bacterial population of the whey after 3 days of storage was reduced from an initial load of 2.1×10^5/ml to 117/ml respectively. Furthermore, at these levels of sodium sulfite no bacterial growth was observed after 10 days of storage. But, at lower levels of sodium sulfite, i.e. 0.2% and below, it was not effective as a chemical preservative, since the increase in bacterial count could not be arrested. In the above instances, the treatment was carried out at pH 5.0, the original pH of the whey.

The effect of varying pH on the effectiveness of sodium sulfite as a preservative is presented in Table 5. To study the effect of varying pH, the whey was treated with 0.4% sodium sulfite at pH ranging from 3.5 to 7.0.

TABLE 4. The effect of sodium sulfite concentration on the viable bacterial cells of the whey preserved at its original pH, 5.0, during storage at 70-72°F.

Sodium sulfite %	Initial pH	Viable bacterial cells per ml (Average of 2 trials)			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	2.1×10^5	9.1×10^7	*16.7×10^8	*7.8×10^7
0.10	"	"	8.2×10^6	3.7×10^8	5.1×10^{10}
0.20	"	"	4.1×10^2	5.9×10^3	8.9×10^4
0.30	"	"	11.7×10^1	0.0	0.0
0.40	"	"	2.5×10^1	0.0	0.0

* Slight mold growth

Appendix Tables: 5, 6, 7

TABLE 5. The effect of 0.40% sodium sulfite on the viable bacterial cells of the whey preserved at pH ranging 3.5-7.0, during storage at 70-72°F (Average of 2 trials)

Sodium sulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	2.1×10^5	4.4×10^7	9.0×10^7	*14.1×10^8
0.40	3.50	"	0.0	0.0	0.0
"	4.40	"	0.0	0.0	0.0
"	5.00	"	2.5×10^1	0.0	0.0
"	6.00	"	7.8×10^4	16.0×10^7	2.3×10^8
"	7.00	"	12.7×10^8	8.0×10^9	*19.6×10^8

* moldy

Appendix Tables: 8, 9, 10.

Treatment of the whey with 0.4% sodium sulfite at pH 5.0 and below, showed effective preservation, as the original viable cell count of 2.1 x 10^5/ml was reduced to zero and maintained so, up to 21 days of storage. However, at pH 4.4 and below, the treatment was more effective than the treatment at pH 5.0. In the former case the original cell count of 2.1 x 10^5/ml was reduced to zero within the first 3 days, whereas at pH 5.0 it was reduced to 25/ml in the first 3 days and required 10 days to reduce the bacterial population to zero. Treatment with 0.4% sodium sulfite at pH 6.0 and 7.0 had no preservative effect at all, as it could neither reduce the original bacterial content nor could it prevent further multiplication.

The changes in the pH of the whey when treated with sodium sulfite, during storage at 70-72^{0}F are presented in Appendices 11 and 12.

The whey samples treated with 0.2-0.4% sodium sulfite at pH 3.5-5.0 maintained their pH during storage, whereas the rest of the samples showed greater variation in pH during storage.

The decline in lactose content of the whey treated with sodium sulfite at various pH ranging from 3.5-7.0, is shown in Figure 6 and Appendix Tables 13-16. Treatment of the whey with 0.4% sodium sulfite at pH 3.5-4.4 showed

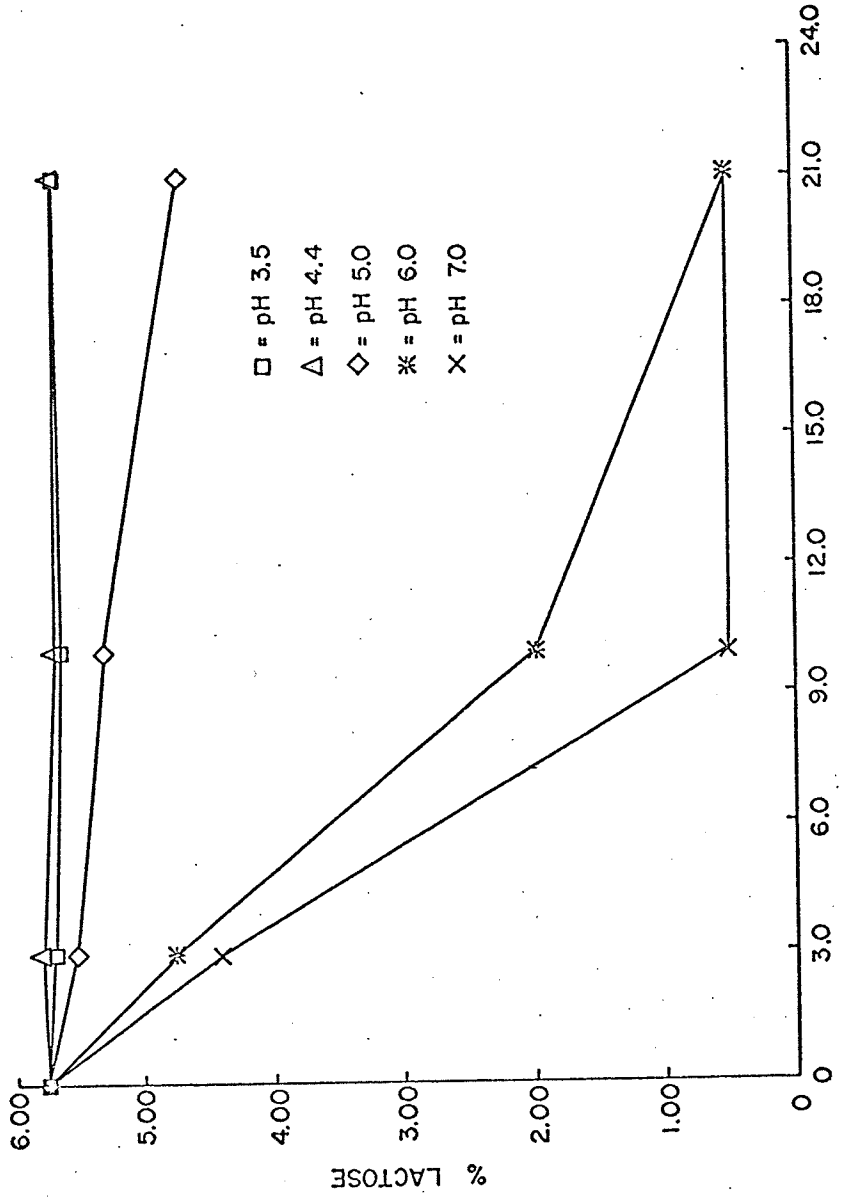


Fig.6 The Effect of pH on Lactose Content of Whey at 0.4% Sodium sulfite During Storage at 70-72° F

no decline in lactose content of the whey throughout storage of 21 days. Also the lactose content of the whey treated with 0.4% sodium sulfite at pH 5.0 showed no significant drop in the first 10 days. However, when storage was further extended up to 21 days the lactose content was reduced by about 15%. Treatment of the whey at pH 6.0 and 7.0 showed a very sharp decline in lactose levels, and at the end of 21 days of storage, the lactose contents were reduced from the original 5.76% to 0.5% end below.

It is evident from the above results that the treatment of the whey with 0.3-0.4% sodium sulfite at pH 3.5-4.4 was very effective in preservation of the whey. It was able to reduce the original bacterial level of 2.1×10^5/ml to zero, and maintained this condition during storage up to 21 days. The samples preserved by this method showed no significant changes in either the pH or in the lactose content of the whey during storage.

(iii) The Effectiveness of Sodium Metabisulfite on the Preservation of the Whey.

Preliminary data (Table 3, Figure 5) revealed that 0.1% sodium metabisulfite was effective in lowering the original bacterial population of 9.4×10^3/ml to 42/ml during 7 days of storage.

Based on the above results, different concentrations of sodium metabisulfite were used to determine its effectiveness at pH ranging from 3.5 to 7.0. The results presented in Table 6 indicate that sodium metabisulfite at levels of 0.15% and higher was able to reduce the original bacterial population of 2.1×10^5/ml to zero, and maintained this condition up to 21 days of storage. It was found that 0.3% of sodium metabisulfite was most effective, as it could reduce the original bacterial level of 2.1×10^5/ml to zero within the first 3 days of storage. Sodium metabisulfite concentrations of 0.15% and 0.2% were relatively less effective. In these instances the original bacterial count of 2.1×10^5/ml, though considerably reduced in the first 3 days, was found to have dropped to zero when observed after 10 days of storage.

The effect of varying pH on the effectiveness of sodium metabisulfite as a preservative is presented in Table 7. In order to study the effects of varying pH, the whey was treated with 0.15% sodium metabisulfite at pH ranging from 3.5-7.0. Treatment of the whey at pH 3.5 and 4.4 effectively preserved the whey by reducing the original bacterial count of 2.1×10^5/ml to zero, and by maintaining this condition up to 21 days of storage. However, the same level of sodium metabisulfite (i.e. 0.15%)

TABLE 6. The effect of sodium metabisulfite concentration on the viable bacterial cells of the whey preserved at its original pH, 5.0, during storage at 70-72°F (Average of 2 trials).

Sodium metabisulfite %	Initial pH	Viable bacterial cells per ml.			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	2.1×10^5	9.1×10^7	*16.7×10^8	*7.8×10^7
0.05	"	"	7.1×10^3	*	*
0.10	"	"	4.7×10^3	*	*
0.15	"	"	3.3×10^3	0.0	*0.0
0.20	"	"	3.7×10^1	0.0	0.0
0.30	"	"	0.0	0.0	0.0

* moldy

Appendix Tables: 17, 18, 19.

TABLE 7. The effect of 0.15% sodium metabisulfite on the viable bacterial cells of the whey preserved at pH ranging from 3.5-7.0, during storage at 70-72°F. (Average of 2 trials).

Sodium metabisulfite %	Initial pH	Viable bacterial cells per ml.			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	2.1×10^5	4.4×10^7	9.0×10^7	$*14.1 \times 10^8$
0.15	3.50	"	0.0	0.0	0.0
"	4.40	"	0.0	0.0	0.0
"	5.00	"	3.3×10^3	0.0	*0.0
"	6.00	"	4.0×10^6	$*4.7 \times 10^7$	$*16.2 \times 10^8$
"	7.00	"	6.7×10^6	$*5.7 \times 10^7$	$* 4.9 \times 10^9$

* moldy

Appendix Tables: 20, 21, 22.

was not effective at pH 6.0-7.0 as a preservative. Since it could not effectively reduce the original bacterial count of 2.1 x 10^5/ml and nor could it prevent the growth of mold and bacteria.

The changes in the pH of the whey, treated with sodium metabisulfite, during storage at 70-72°F are presented in Appendices 23, and 24. The whey samples which were treated with sodium metabisulfite at pH 3.5-5.0 showed no significant changes in the pH during storage. However, the whey samples which either had no chemical treatment (i.e. control) or those which were treated with sodium metabisulfite at pH 6.0 and 7.0, showed considerable variations in pH during storage.

The effect of pH in maintaining the original lactose content of the whey treated with sodium metabisulfite as a preservative, during storage at 70-72°F is indicated by Figure 7 and Appendix Tables 25-28. The whey treated with 0.15% sodium metabisulfite at pH 3.5 and 4.4 showed no decline in the lactose contents during storage of 21 days. Treatment of the whey with 0.15% sodium metabisulfite at pH 5.0 showed about 30% decline in lactose content of the whey at the end of 21 days of storage. Whereas treatment of the whey with above concentration of sodium metabisulfite (i.e. 0.15%) at pH 6.0 and 7.0 showed a very sharp decline in the lactose content of the whey,

and within 10 days of storage, about 90% of the lactose was depleted.

It appeared from the above that treatment of the whey with 0.15% sodium metabisulfite at pH 4.4 and below, was very effective in extending the storage life of the whey up to 21 days. It could reduce the original bacterial count of 2.1×10^5/ml to zero, and maintained this level throughout the storage of 21 days. It also prevented changes in the pH during storage, and maintained the lactose conent of the whey at its original level.

(iv) The Effectiveness of Hydrogen Peroxide on the Preservation of the whey.

Preliminary investigation (Table 3, Figure 5) revealed that 125 p.p.m. of hydrogen peroxide was able to prevent an increase in the original bacterial population of the whey (9.4×10^3/ml) during 7 days of storage. However, at this concentration of hydrogen peroxide, it was not effective in lowering the bacterial population. The bacterial population of the whey, when treated with 250 p.p.m. or 500 p.p.m. of hydrogen peroxide was reduced to zero. In these instances, the treatment was carried out at pH 5.25, the original pH of the whey.

Based on the above results, further treatment of whey was carried out with 250 p.p.m. of hydrogen peroxide

at pH ranging from 3.5 - 7.0, in order to establish the optimum conditions for preservation. Table 8 presents the effect of hydrogen peroxide (250 p.p.m.) treatment at varying pH, on the bacterial count of the whey. The treatment of the whey with 250 p.p.m. hydrogen peroxide at pH 3.5 and 4.4 proved very effective, as the original bacterial load of 3.8×10^4/ml was reduced to zero, and could be maintained so for 21 days. Treatment of the whey with 250 p.p.m. of hydrogen peroxide at pH 5.0 and above was not effective in preserving the whey. It showed no bactericidal or inhibitory effect under these conditions.

The changes in the pH of the whey samples, treated with 250 p.p.m. hydrogen peroxide, during storage at 70-72°F are presented in Appendix 32. The whey samples treated at pH 3.5 and 4.4 showed minor changes in pH during storage. The variation was downward and ranged from 0.05 to 0.4. The whey samples which were treated at pH 5.0 and above, showed considerably high degree of variation in the pH with the magnitude of about $\pm$ 2.

The decline in the lactose contents of the whey samples treated with 250 p.p.m. hydrogen peroxide at pH ranging from 3.5 - 7.0 is presented in Figure 8, and Appendix Tables 33 and 34. Treatment of the whey at pH 3.5 and 4.4 showed no decline in the lactose contents of the whey throughout 21 days of storage, However, treatment

TABLE 8. The effect of 250 p.p.m. hydrogen peroxide on the viable bacterial cells of the whey preserved at pH ranging 3.5-7.0, during storage at 70-72°F. (Average of 2 trials).

Hydrogen peroxide	Initial pH	Viable bacterial cells per ml.			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	3.8×10^4	5.6×10^6	4.8×10^8	*1.2×10^9
250 p.p.m.	3.50	"	0.0	0.0	0.0
"	4.40	"	13.2×10^1	0.0	0.0
"	5.00	"	10.6×10^4	*3.1×10^6	*7.2×10^6
"	6.00	"	10.2×10^6	*5.0×10^6	*6.2×10^6
"	7.00	"	13.1×10^7	*10.9×10^6	*9.1×10^6

* moldy

Appendix Tables: 29, 30, 31.

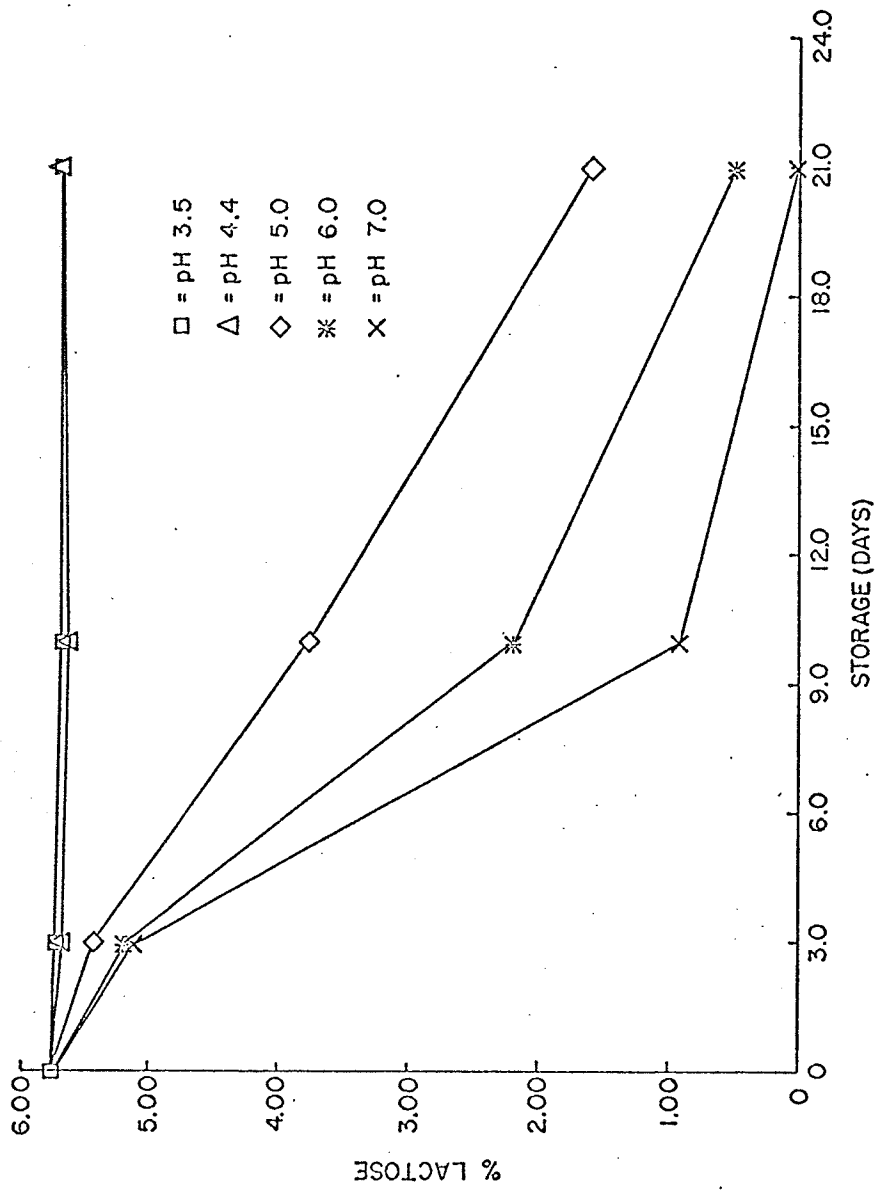


Fig. 8 The Effect of pH on Lactose Content of Whey at 250 p.p.m. Hydrogen peroxide During Storage at 70-72°F

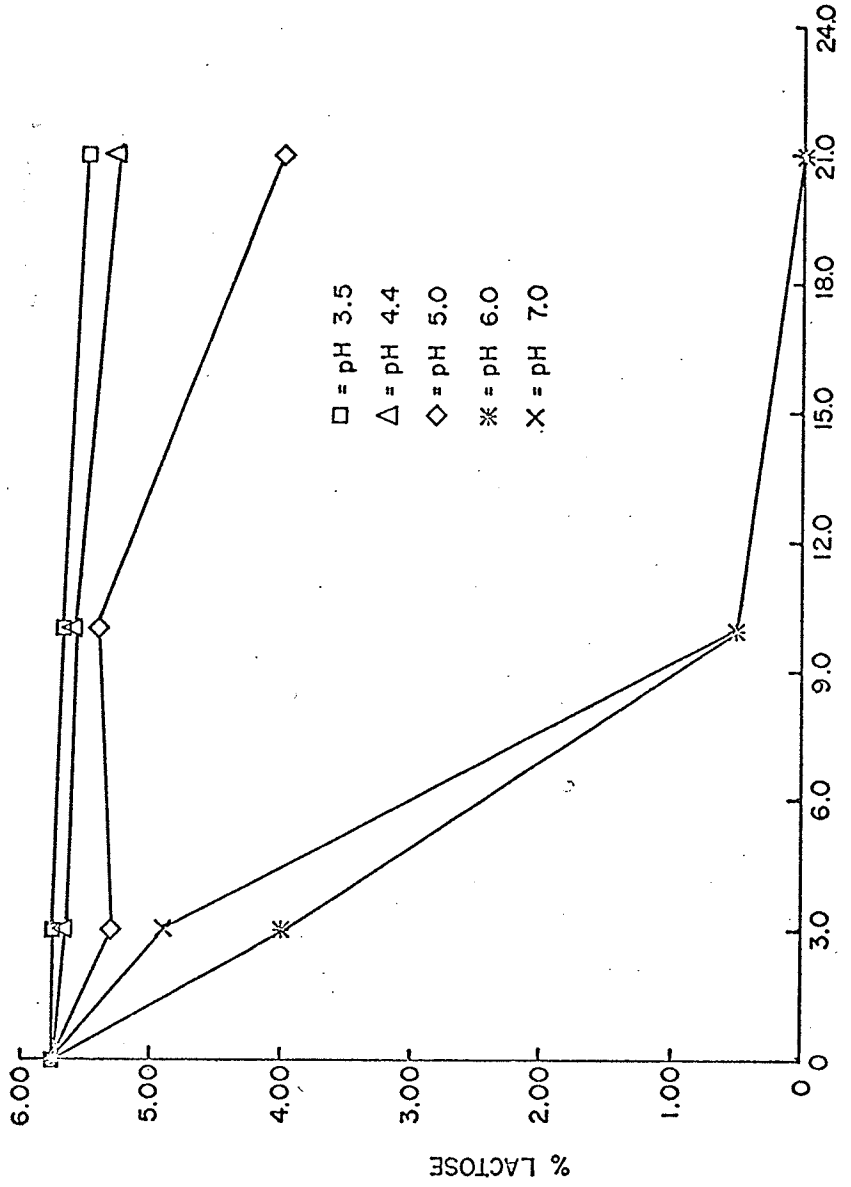


Fig.7 The Effect of pH on Lactose Content of Whey at 0.15% Sodium metabisulfite During Storage at 70-72°F

at pH 5.0, 6.0, and 7.0 showed a decline in lactose contents of the whey by about 6-10% in first 3 days, which was followed by a very rapid drop in the lactose contents, as the storage time was extended up to 21 days.

PART II: YEAST PROPAGATION

Investigations were carried out to establish the optimum conditions for the growth of Torula cremoris and Saccharomyces fragilis with respect to pH, temperature, nutrient supplementation, effect of aeration, size of inoculum, and heat treatment. In addition, studies on the suitability of chemically preserved whey for the propagation of Torula cremoris and Saccharomyces fragilis were carried out.

In subsequent discussions the term "whey", unless otherwise specified, stands to mean the fresh whey heat-treated at 104°C for 15 minutes.

(1) The Effect of pH on the Growth of Yeast

Results in Part I (i.e. Preservation of the whey by chemical preservatives) have shown that pH 5.0 and below was relatively effective from the point of view of preservation. Earlier it had been suggested that the pH 4.4 was suitable for the growth of Torula cremoris (Graham et al.,1953). Therefore, no attempts were made

to study the growth pattern of Torula cremoris under various pH conditions. Instead, the present investigation was designed to determine whether the growth pattern of Torula cremoris was different at pH 4.4 and 5.0. In the event that if the growth pattern was not different, then the whey preserved at pH 5.0, could be directly used for fermentation without readjusting the pH to 4.4.

Table 9 summarizes the growth pattern of Torula cremoris when grown in the Model I fermenter and with the pH of the whey adjusted to 4.4 and 5.0 respectively. During the course of fermentation, the change in the pH was adjusted back by addition of acid or alkali. As is evident from the results (Table 9), the utilisation of lactose by Torula cremoris was relatively rapid at pH 4.4. At the end of 12 hours of fermentation, the amount of lactose utilized was 100% and 85% at pH 4.4 and 5.0 respectively.

The yield based on cell count per ml, at the end of 12 hours of fermentation was about 8% higher at pH 4.4 than at pH 5.0. It suggested that the rate of utilization of lactose was relatively more rapid at pH 4.4 than at pH 5.0.

The optimum pH for the growth of Saccharomyces fragilis has been reported to be 5.0-5.7 (Wasserman, 1960, Wasserman et al., 1961). Therefore in the present study no effort

TABLE 9. Growth of *Torula cremoris* in whey at pH 4.4 and 5.0, and temperature 28°C.

pH		Hours 0	4	8	12
4.40	Yeast count (per ml x 10^6)	125.0	312.5	625.5	605.0
	pH	4.40	*4.40	4.50	4.50
	Lactose %	2.80	1.62	0.30	0.00
5.00	Yeast count (per ml x 10^6)	125.00	295.00	530.00	564.00
	pH	5.00	*5.05	5.10	5.15
	Lactose %	2.80	1.78	0.58	0.50

* pH brought down to near original by addition of acid.

was made to investigate the effect of various pH values on the growth of Saccharomyces fragilis. pH 5.0-5.7 was adopted as optimum for the growth of Saccharomyces fragilis.

(ii) The Effect of Nutrient Supplementation on the Growth of Yeast.

The effect of nutrient supplementation on the growth of Torula cremoris in the Model II fermenter is presented in Table 10. The fermentation was carried out by the use of inoculum Type B, maintaining the medium at pH 4.4, and at 28°C.

In the nutrient supplemented whey, the growth of Torula cremoris and the corresponding rate of lactose disappearance, was more rapid than that in non-supplemented whey. This resulted in a shorter time being required for complete utilization of lactose and also gave higher yield of yeast cells in nutrient supplemented whey. In 18 hours of fermentation, in the whey supplemented with 0.25% urea plus 0.25% diammonium phosphate, 100% of the lactose was utilized with a resultant yield of about 1,000 x 10^6 cells/ml. Whereas in non-supplemented whey about 90% of the lactose was utilized in 18 hours of fermentation, and the resultant yield was only about 70% as compared to nutrient supplemented whey.

TABLE 10. The effect of nutrient supplementation on the growth of *Torula eremoris* in Model II fermenter.

Nutrient supplementation		Hours						
		0	4	8	12	16	18	22
0.25% urea + 0.25% diammonium phosphate	Yeast count (per ml x 10^6)	62.5	109.0	236.0	-	910.0	1008.0	-
	Lactose %	4.52	4.25	3.70	-	0.50	0.0	-
None	Yeast count (per ml x 10^6)	62.5	80.0	140.0	252.0	480.0	690.0	745.0
	Lactose %	4.60	4.20	3.60	2.70	1.00	0.50	0.0

(iii) The Effect of Temperature on the Growth of Yeast.

The effect of temperature on the growth of Torula cremoris in the whey containing 0.25% urea and 0.25% diammonium phosphate, is summarized in Table 11. The propagation was carried out in the Model I fermenter at 26, 28, and 30°C temperatures respectively. The pH during fermentation was maintained at 4.4.

The results indicated that Torula cremoris was able to multiply relatively faster at 28-30°C, than at 26°C. At temperature of 28-30°C the organisms reached their maximum growth within 8 hours. However, at 26°C it took longer (10 hours) to reach the same level of cell count.

The optimum temperature for the growth of Saccharomyces fragilis has been reported to be 31-33°C by many workers (Wasserman, 1960 a,b; and Burdock et al., 1967). In the present investigation 32 ± 1°C was adopted for the growth of Saccharomyces fragilis, and no attempt was made to study the growth pattern of Saccharomyces fragilis at various temperatures.

(iv). The Effect of the Size of Inoculum

The results showing the effect of the size of inoculum on the growth of Torula cremoris are presented

TABLE 11. Growth of Torula cremoris in the whey containing 0.25% urea plus 0.25% diammonium phosphate at pH 4.4, and temperature 26, 28 and 30°C.

Temperature		Hours				
		0	4	8	12	13½
26°C.	Yeast count (per ml x 10^6)	65.0	114.0	380.0	450.0	472.00
	Lactose %	4.25	3.80	0.85	0.55	0.32
28°C.	Yeast count (per ml x 10^6)	65.0	118.0	465.0	498.0	489.0
	Lactose %	4.25	3.20	0.44	0.15	0.0
30°C.	Yeast count (per ml x 10^6)	65.0	110.0	418.0	424.0	442.0
	Lactose %	4.25	3.50	0.52	0.38	0.0

in Table 12. The organism was grown in the Model II fermenter at pH 4.4 and temperature 28°C.

Under conditions of the experiment with an initial yeast count of 62.5 x 10^6/ml (obtained by 5% inoculum by volume), it required 15 hours of fermentation to reach the maximum level of about 1,000 x 10^6 yeast cells per ml. Increasing the size of inoculum to 15% (by volume), gave an initial cell count of 200 x 10^6/ml, and about the same level of yeast yield was achieved within 11 hours of fermentation.

(v). The Effect of Aeration

Table 13 summarizes the effect of aeration on the growth of Torula cremoris in the fermenter Model II. Other factors included the use of inoculum Type B; supplementation with 0.25% urea plus 0.25% diammonium phosphate; the pH, 4.4; and the temperature, 28°C.

Under the conditions of experiment it required 18 hours to attain peak growth of 1,000 x 10^6 yeast cells/ml at an O.A.R. (Oxygen Absorption Rate) value of 2.2 mMO_2/l/min. But when the O.A.R. was increased to 3.5 mMO_2/l/min., it required only 15 hours to reach the growth peak of a similar magnitude.

TABLE 12. The effect of size of inoculum on the growth of *Torula cremoris* in whey containing 0.25% urea plus 0.25% diammonium phosphate, at pH 4.4 and temperature 28° C.

	Hours						% Efficiency	
	0	4	8	11	12	15	Lactose utilization	C.O.D. reduction
Yeast count (per ml x 10^6)	62.5	118.0	290.0	-	550.0	1,015.0		
Lactose %	4.50	3.50	2.55	-	1.00	0.0	65.7	76.0
Soluble C.O.D. (mg/l)	47,828.0					11,508		
Yeast count (per ml x 10^6)	200.0	400.0*	865.0**	1,107.0	985	-		
Lactose %	3.47	1.60	0.30	0.0	0.0	-	69.7	79.0
Soluble C.O.D. (mg/l)	41,212.8				8,359.2			

* 6 hours
** 8½ hours

TABLE 13. The effect of aeration on the growth of *Torula cremoris* in whey containing 0.25% urea + 0.25% diammonium phosphate, in Model II, fermenter.
(pH 4.4, temperature 28° C, and inoculum Type B).

O.A.R. ($mMO_2/l/min.$)		Hours 0	4	8	15	16	18	% Efficiency Lactose utilization	% Efficiency C.O.D. reduction
	Yeast count (per $ml \times 10^6$)	62.5	109.0	236.0		910.0	1,008		
2.20	Lactose %	4.52	4.25	3.70		0.50	0.0	59.0	74.7
	C.O.D. (mg/l)	48,216	-	-	-	-	12,152		
	Yeast count (per $ml \times 10^6$)	62.5	118.0	290.0	1,015.0	1,028.0	-		
3.50	Lactose %	4.50	3.50	2.55	0.0	0.0	-	65.7	76.0
	C.O.D. (mg/l)	47,828				11,508			

(vi). Fermenter Design

The growth pattern of Torula cremoris in the whey containing 0.25% urea plus 0.25% diammonium phosphate in the Model I and Model II fermenters, is summarized in Table 14. Other factors included the use of inoculum Type B; the pH, 4.4; and the temperature, 28°C.

The rate of disappearance of lactose was high in the fermenter Model I, and within 12 hours of fermentation almost all the lactose was utilized with a resulting yield of about 600 x 10^6 yeast cells per ml of fermented whey. But in the fermenter Model II though it required 16-18 hours to utilize an equal quantity of lactose, the resultant yield of 1,000 x 10^6 yeast cells per ml was much higher than that of the first instance.

(vii). The Effect of Heat Treatment

Results on the effect of heat treatment on the growth of Torula cremoris in whey containing 0.25% urea plus 0.25% diammonium phosphate are summarized in Table 15. During fermentation, the medium was maintained at pH 4.4; temperature, 28°C; and O.A.R., 3.5 mMO_2/l/min.

Under similar conditions of propagation the rate of lactose utilization and the resultant yield of yeast cells in the case of raw whey was much lower when compared to heat treated whey. In heat treated whey, the yeast

TABLE 14. Growth of *Torula cremoris* in whey containing 0.25% urea plus 0.25% diammonium phosphate, in Model I and Model II fermenters, at pH 4.4, temperature 28°C, and inoculum Type B.

Fermenter		Hours					
		0	4	8	12	16	18
Model I	Yeast count (per ml x 10^6)	65.0	120.0	480.0	585.0	605.0	600.0
	Lactose %	4.48	3.50	1.08	0.30	0.0	0.0
Model II	Yeast count (per ml x 10^6)	62.50	10.90	236.0	-	910.0	1,008.0
	Lactose %	4.52	4.25	3.70	-	0.50	0.0

TABLE 15. The effect of heat treatment on the growth of Torula cremoris in whey containing 0.25% urea plus 0.25% diammonium phosphate (pH 4.4, temperature 28°C, inoculum Type B, and O.A.R. = 3.5 $mMO_2/l/min.$)

Heat treatment		Hours 0	Hours 11	Hours 22
Raw whey (i.e. no heat treatment)	Yeast count (per ml x 10^6)	200.0	*750.0	1,285.0
	Lactose %	2.38	1.18	0.0
104°C for 15 minutes	Yeast count (per ml x 10^6)	200.0	1,107.0	-
	Lactose %	3.47	0.0	-
70°C. for 5 minutes	Yeast count (per ml x 10^6)	200.0	1,250.0	-
	Lactose %	3.60	0.0	-

* 11½ hours

cells required 11 hours to reach peak growth, whereas in the case of unheated whey, the time of fermentation was as high as 22 hours for the yeast cells to reach peak growth.

(viii). The Effect of Chemical Preservation on Yeast Growth.

Table 16 summarizes the growth pattern of Torula cremoris and Saccharomyces fragilis in H_2O_2 preserved whey. The residual hydrogen peroxide was decomposed by addition of catalase, and further it was heat treated at 70°C for 5 minutes. Other factors during fermentation included the utilization of inoculum, Type B; O.A.R., 3.5 mMO_2/l/min.; supplementation by 0.25% urea plus 0.25% diammonium phosphate; and the use of the fermenter Model II. For Torula cremoris and Saccharomyces fragilis the pH was kept at 4.4 and 5-5.5; and the temperature was maintained at 28°C and 32°C respectively.

From the results shown in Tables 15 and 16, it appeared that there was no significant difference in yields of Torula cremoris between H_2O_2 preserved and non-preserved whey.

Under similar conditions of nutrient supplementation, size of inoculum and aeration rate, Saccharomyces fragilis appeared to give relatively higher yeast yield than Torula cremoris. However, the time of fermentation necessary

TABLE 16. Growth of Torula cremoris and Saccharomyces fragilis in H_2O_2 preserved whey, after decomposition of residual hydrogen peroxide by catalase, followed by heat treatment at 70°C for 5 minutes.

Organism		Hours 0	4	8	10	11	Lactose Utilised g/l	Centrifuged Solids g/l	% Efficiency of Lactose Utilisation
T.cremoris	Yeast count (per ml x 10^6)	200.0	325.0	690.0	880.0	1,180.0	38.2	14.298	68
	Lactose %	3.82	3.35	1.64	0.60	0.0			
S.fragilis	Yeast count (per ml x 10^6)	200.0	380.0	850.0	1,240.0	1,374.0	36.0	14.653	74
	Lactose %	3.60	3.20	1.80	-	0.0			

for peak growth and lactose utilization did not seem to be different in both cases.

The whey chemically preserved by 0.15% sodium metabisulfite or 0.3% sodium sulfite at pH 5.0 could not support the growth either of Torula cremoris or that of Saccharomyces fragilis. No increase in initial yeast count was observed during fermentation. It suggested that these concentrations of sulfur salts, though efficient from the point of view of preservation, left undecomposed residue of sulfurous acid which apparently inhibited the growth of yeast.

DISCUSSION

In this study an attempt was made to investigate the preservative effect of various chemicals on whey and the suitability of such chemically preserved whey for yeast production.

Preliminary work performed in this study indicated that sodium sulfite, sodium metabisulfite and hydrogen peroxide at pH 5.25 (the original pH of the whey) were effective in preserving whey. Further investigations examined the suitability of the above chemicals with respect to the optimum pH level for preservation, and the associated changes in the chemical and bacteriological content of the whey during storage up to 21 days at room temperatures (70-72°F).

Whey was more effectively preserved with 0.3 - 0.4% sodium sulfite, 0.15 - 0.3% sodium metabisulfite or 250 p.p.m. hydrogen peroxide at pH 3.5 - 4.4, than at higher pH levels. In all instances increasing H^+ ion concentration was found to increase the effectiveness of chemical preservation. It is evident that the pH of the whey strongly influenced the growth inhibiting and/or bactericidal properties of these chemicals. Earlier the similar role of pH in the effectiveness of HSO_3^- as an antimicrobial agent was reported by Cruess et al. (1952,1953, 1954) and Furia (1968). Cruess et al. reported that at

pH 3.5, two to four times as much sulphur dioxide was needed to inhibit microbial growth as compared to that at pH 2.5. At pH 7.0, SO_2 was ineffective against yeasts and molds, and 1,000 p.p.m. was required to inhibit bacteria. The effectiveness of preservation at increasing H^+ ion concentration was observed also for benzoic acid (Smith et al., 1962).

The enhanced effectiveness of preservation on treatment with sulfites at low pH, is thought to be the result of more extensive penetration of the cell wall by un-ionized sulfurous acid. Further, it seems that sulfurous acid may block enzymes of the microorganisms by reducing essential disulfide (-S-S-) linkages (Wyss, 1948). Behm (1964) studied the antimicrobial activity of sulfurous acid on Saccharomyces cerevisiae and Escherichia coli, and concluded that the steps in respiratory mechanisms which involve nicotinamide dinucleotide, are inhibited as a result of formation of certain hydroxysulfonates formed by combinations of SO_2 with ketone groups. The increased efficiency of preservation by hydrogen peroxide in a low pH range is thought to be the result of irreversible denaturation of cellular enzymes (Wyss, 1948; Smith et al., 1962).

In the preservation of the whey with 0.4% sodium sulfite, or 0.15% sodium metabisulfite, or 250 p.p.m.

hydrogen peroxide at pH 3.5-4.0 there was no decline in the lactose content of the whey during storage up to 21 days. When the treatment was carried out with these chemical concentrations at pH 6.0-7.0, the lactose content was depleted by about 95% during storage for 21 days. This would appear to follow previous observations.

Concentrations of sodium sulfite below 0.3%, sodium metabisulfite below 0.15%, and hydrogen peroxide below 250 p.p.m., were not effective in preserving the whey in that there some increase in bacterial population. Despite this fact however, there was no decline in the lactose content of the whey for the first 3 days. This can be explained on the basis that the microbial cells, originally present in the whey, could survive and grow at the expense of lactic acid initially present in the whey. After this period the lactose content dropped sharply.

The above results have demonstrated that the H_2O_2 treatment in relatively low concentration was more effective in preservation of whey than the sodium sulfite or sodium metabisulfite treatments. The study also showed that a pH of 4.4 was relatively suitable for the growth of Torula oremoris than a pH of 5.0. This observation confirmed the earlier findings of Graham et al. (1951). The effectiveness of pH 4.4 for the growth of Torula oremoris was associated with the fast rate of lactose utilization and higher yeast yields.

Although whey contains compounds of nitrogen, phosphorus, and other nutrients; supplementation has been found to be necessary for higher yeast yields. Wasserman (1960) reported that the supplementation of whey with 0.225% phosphorus and 0.13% nitrogen in suitable salts was adequate to provide optimum yields of Saccharomyces fragilis. Taking the above into consideration, lactose-yeast extract broth was used for making inoculum and the whey medium was supplemented with 0.25% urea plus 0.25% ammonium phosphate. The addition of 0.25% urea plus 0.25% ammonium phosphate resulted in increasing the yeast yield by about 40% over non-supplemented whey. With regard to temperature requirements Graham et al. (1951) reported that 26°C was suitable for the growth of Torula cremoris. However, results in this study indicated that a temperature of 28-30°C was relatively more suitable for the growth of Torula cremoris. The rate of lactose utilization and the resultant yeast yield were relatively higher at 29-30°C. In addition adequate oxygen supply has been reported to be necessary for optimum yeast yields (Wasserman 1960). In this study an increase in the oxygen absorption rate (O.A.R.) from 2.20 $mMO_2/l/min.$ to 3.50 $mMO_2/l/min.$ reduced the time of fermentation considerably. Under maximum possible air flow, the oxygen absorption rate was increased from 2.55 $mMO_2/l/min.$ to

3.50 $mMO_2/l/min.$ by the installation of impellers driven by a high speed motor. This resulted in increased oxygen absorption efficiency in two ways, namely; (i) by breaking the foam effectively which in turn could minimize the need to add antifoam; and (ii) by dispersion of the air bubbles which in turn facilitated distribution and absorption of air into the fermentation medium. In the present study 3.5 $mMO_2/l/min.$ was the highest level that could be achieved in the fermenter Model II. Wasserman et al., 1961 reported that optimum oxygen level for yeast propagation was 4.75 $mMO_2/l/min.$ This observation indicated that the oxygen absorption efficiency to a great extent depended on the design of the apparatus.

The present investigation indicated that Torula cremoris when grown in the Model I fermenter, depleted the lactose content after 12 hours of fermentation. In the Model II fermenter although 18 hours of fermentation was required, the resultant yield was about 40% higher than the former case. The rapid rate of lactose disappearance in the Model I fermenter could be attributed to the conversion of a considerable portion of lactose into CO_2 and other metabolites instead of its complete utilization to form yeast cells. This phenomenon was probably due to relatively less efficient aeration and air dispersion in

the Model I fermenter than that of the Model II fermenter.

With respect to pretreatment of whey, it was found that mild heat treatment of whey resulted in complete utilization of the lactose content by Torula cremoris and the attainment of the growth peak within 11 hours of fermentation. Unheated whey required twice as much time to attain the same conditions. Earlier it had been reported that the heat treatment of the whey resulted in improved yeast yields (Emebo et al.,1942; Hanson et al.,1949). In contradiction to this, Wasserman (1960) reported that there was no difference in yeast yields for heat treated and non-heat treated whey. However, the results of this study appeared to support the results of Emebo et al. (1942) and Hanson et al. (1949). The heat treatment presumably rendered the whey medium relatively more suitable for yeast growth by the presence of certain nitrogenous growth nutrients as a result of protein degradation. Furthermore, it is possible that lactose in part, was hydrolyzed in heat treated whey, liberating glucose and galactose which were readily utilized by yeasts.

Earlier it was demonstrated that treatment of the whey with 250 p.p.m. hydrogen peroxide at pH 3.5-4.4 was effective in preserving the whey up to 21 days. It was observed that in the whey samples treated with 200-250 p.p.m.

hydrogen peroxide when stored for 3 days and longer, only about 125 p.p.m. hydrogen peroxide had decomposed or combined with the whey constituents and the remainder was present in the whey as residue. The presence of this residual hydrogen peroxide appeared to be necessary to suppress microbial activity during storage. The residual hydrogen peroxide was decomposed by the addition of the enzyme catalase, followed by heat treatment at 70°C for 5 minutes. The activity of catalase in buffer medium at pH 7.0 and temperature 28°C was found to be 1,430 sigma units per mg where 1 sigma unit was equated to the decomposition of 1 micro mole of H_2O_2 per minute (sigma, 1969). Based on this activity, 1.65 mg of enzyme preparation should be able to decompose 80 p.p.m. of hydrogen peroxide in 1 liter of buffer medium per minute at pH 7.0 and temperature 28°C. However, results of this study indicated that 8.5 mg of the enzyme preparation was required to decompose 80 p.p.m. of hydrogen peroxide residue in 1 liter of whey at pH 4.4. and temperature 28°C in 45 minutes. This indicated that the activity of the enzyme in whey medium at pH 4.4 was much less than that in the buffer medium at pH 7.0. Since the whey treated with hydrogen peroxide at pH 4.4 was required for optimum growth of yeast, it was not adjusted any further. It is difficult

to explain the loss in the activity of the enzyme except that to an extent it was apparently due to pH 4.4 of the whey medium instead of pH 7.0 which is optimum for the enzyme activity. Also there might be some organic substances such as short chain alcohols etc., in whey, that act as hydrogen donors and could conceivably reduce catalase activity. This observation was also reported by Reed, 1966.

Whey which was chemically preserved with 0.15% sodium metabisulfite or 0.3% sodium sulfite did not support the growth of either of the test organisms. No increase in initial yeast count was observed during fermentation. It suggested that the above levels of sodium metabisulfite and sodium sulfite although very effective for preservation, apparently left undecomposed residue of sulfurous acid which inhibited the growth of yeast species.

Finally this study indicated that there was no difference in the growth pattern of Torula cremoris in fresh whey and in H_2O_2 preserved whey, when used as fermentation medium. A comparison of Torula cremoris and Saccharomyces fragilis in this study indicated that Saccharomyces fragilis had a relatively higher lactose utilization efficiency.

SUMMARY

The literature about the chemical preservation and utilization of whey for yeast propagation has been reviewed, and investigations on preservation of whey and its suitability for yeast propagation have been presented.

The treatment of whey at pH 3.5 - 4.4 with 0.3% sodium sulfite, or 0.15% sodium metabisulfite, or 250 p.p.m. hydrogen peroxide was found to extend the storage life of whey up to 3 weeks when stored at 70-72°F under sterile conditions.

Torula cremoris was found to be best grown at pH 4.4 and at 28°C. At deviations from the above, the rate of lactose utilization and the corresponding growth rate, were relatively poor. For the propagation of Saccharomyces fragilis pH 5 - 5.5, and 32°C were considered suitable.

Addition of 0.25% urea plus 0.25% ammonium phosphate was able to supplement the whey in inorganic nitrogen and phosphorous so as to provide optimum growth of Torula cremoris and Saccharomyces fragilis.

The mild heat treatment of whey was advantageous, since the whey heat treated for 5 minutes at 70°C was found to be more suitable for the growth of yeast species as compared to raw whey, or the whey heat treated at 104°C for 15 minutes. This could be due the slight heat treatment possibly rendering the protein of whey more readily

utilizable by yeast species.

The whey chemically preserved by 200-250 p.p.m. hydrogen peroxide followed by removal of residual hydrogen peroxide by catalase and then mild heat treatment, was suitable for yeast growth, and the results were comparable with that of non-preserved whey. However, when the whey was preserved with 0.3% sodium sulfite or 0.15% sodium metabisulfite, no yeast growth was observed. This could be explained on the basis that the concentration of sodium sulfite or sodium metabisulfite, which are found to be very effective for the preservation of whey up to 3 weeks, gave a residual level which was too high for yeast propagation.

Preservation of whey by hydrogen peroxide and utilization for yeast production is very promising. The propagation of yeast was able to reduce the dissolved C.O.D. by about 75%, with the lactose utilization efficiency being 70-72%. Thus this system has real merit as a means of coping positively with the problems of whey disposal, particularly in Manitoba.

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APPENDIX TABLE 1. Constituents of lactose-yeast extract broth.

Constituent	g/l
*Lactose Broth (Difco Batch No.498450)	13.0
Bacto Lactose (Difco Batch No.523958)	20.0
Yeast Extract (Difco Control No.460544)	5.0
Diammonium phosphate (Analar 03591802)	2.5
Potassium phosphate (J.T. Baker 3246)	2.5

*Lactose Broth Composition: (per 13.0 g)

Lactose = 5.0 g

Bacto peptone = 5.0 g

Beef extract = 3.0 g

Final pH = 5.0

APPENDIX TABLE 2. Chemical Composition of the whey.

Whey Constituents	Lot 1	Lot 2	Lot 3
I. Moisture	93.87%	91.20%	93.25%
II. Total solids	6.13%	8.80%	6.75%
1) Lactose	4.46%	5.20%	4.65%
ii) Protein	0.95%	1.15%	0.93%
iii) Acidity	0.14%	0.13%	0.15%
iv) Ash	-	-	0.58%
v) Fat	-	-	0.15%
III. pH	5.5	5.2	5.3

APPENDIX TABLE 3. The effect of chemical concentrations on the viable bacterial cells of the cheddar cheese whey, after 7 days of storage at room conditions. (Trial 1) (pH 5.25)

Chemical Preservative	Concentration	Viable bacterial cells per ml (Trial 1) Initial	After 7 days
1) Control	0.00	11.8×10^3	12.2×10^6
ii) Sodium sulfite	0.04%	"	4.2×10^5
"	0.10%	"	1.3×10^4
"	0.16%	"	1.9×10^3
"	0.20%	"	3.8×10^2
iii) Sodium meta-bisulfite	0.01%	"	9.0×10^5
"	0.02%	"	9.8×10^5
"	0.04%	"	18.4×10^4
"	0.10%	"	-
iv) Hydrogen peroxide	50 p.p.m.	"	10.8×10^5
"	125 p.p.m.	"	4.8×10^3
"	250 p.p.m.	"	0.0
"	500 p.p.m.	"	0.0

APPENDIX TABLE 4. The effect of chemical concentrations on the viable bacterial cells of the cheddar cheese whey, after 7 days of storage at room conditions (Trial 2) (pH 5.25)

Chemical Preservative	Concentration	Viable bacterial cells per ml (Trial 2) Initial	After 7 days
i) Control	0.00	7.0×10^3	2.0×10^5
ii) Sodium sulfite	0.04	"	2.2×10^6
"	0.10	"	26.8×10^3
"	0.16	"	5.1×10^3
"	0.20	"	4.0×10^2
iii) Sodium metabisulfite	0.01	"	2.1×10^6
"	0.02	"	15.2×10^5
"	0.04	"	3.2×10^5
"	0.10	"	4.2×10^1
iv) Hydrogen peroxide	50 p.p.m.	"	1.4×10^5
"	125 p.p.m.	"	6.2×10^3
"	250 p.p.m.	"	0.0
"	500 p.p.m.	"	0.0

APPENDIX TABLE 5 The effect of sodium sulfite concentration on the viable bacterial cells of the whey preserved at its original pH 5.0, during storage at 70-72°F (Trial 1).

Sodium sulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	1.7×10^5	18.2×10^6	*8.8×10^8	*6.0×10^6
0.10	"	"	11.2×10^5	3.2×10^8	-
0.20	"	"	3.0×10^2	7.1×10^2	5.2×10^4
0.30	"	"	9.4×10^1	0.0	0.0
0.40	"	"	1.3×10^1	0.0	0.0

*Moldy

APPENDIX TABLE 6. The effect of sodium sulfite concentration of the viable bacterial cells of the whey preserved at its original pH 5.0, during storage at 70-72°F (Trial 2).

Sodium sulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	2.5×10^5	16.4×10^7	24.6×10^8	*1.5×10^8
0.10	"	"	15.3×10^6	4.2×10^8	5.1×10^{10}
0.20	"	"	5.2×10^2	5.2×10^2	12.6×10^4
0.30	"	"	1.4×10^2	0.0	0.0
0.40	"	"	3.7×10^1	0.0	0.0

* Slight mold growth

APPENDIX TABLE 7. Analysis of variance of *Table 4: The effect of sodium sulfite concentration on viable bacterial cells of the whey preserved at pH 5.0.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	143.9797	35.9949	6.9056 = F_T^{**}
Blocks	3	3.8789	1.2929	0.2980 = $F_{B\ (n.s.)}$
Error	12	62.5485	5.2123	
Total	19	210.4071		

* Transformation of data: $X^1 = \log (X+1)$

** Significant at 5% and 1% levels.

APPENDIX TABLE 8. The effect of 0.4% sodium sulfite on the viable bacterial cells of the whey preserved at pH ranging from 3.5 - 7.0 during storage at 70-72°F. (Trial 1).

Sodium sulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	1.7×10^5	8.2×10^7	1.6×10^8	2.8×10^9
0.40	3.50	"	0.0	0.0	0.0
"	4.40	"	0.0	0.0	-
"	5.00	"	3.7×10^1	0.0	0.0
"	6.00	"	3.6×10^4	9.1×10^6	4.6×10^7
"	7.00	"	4.1×10^7	1.2×10^9	*

* Moldy

APPENDIX TABLE 9. The effect of 0.4% sodium sulfite on the viable bacterial cells of the whey preserved at pH ranging from 3.5-7.0 during storage at 70-72°F. (Trial 2).

Sodium sulfite %	Initial pH	Vieble bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	2.5×10^5	6.2×10^6	20.2×10^6	$*19.6 \times 10^6$
0.40	3.50	"	0.0	0.0	0.0
"	4.40	"	0.0	0.0	0.0
"	5.00	"	1.3×10^1	0.0	0.0
"	6.00	"	1.2×10^5	3.1×10^8	4.2×10^8
"	7.00	"	2.5×10^9	14.8×10^9	19.6×10^8

APPENDIX TABLE 10. Analysis of variance of Table 5*: The effect of pH on the viable bacterial cells of the whey preserved with 0.4% sodium sulfite at pH 5.0.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	194.9953	48.7488	8.5936 = F_T n.s.
Blocks	3	11.8969	3.9656	6.9907 = F_B **
Error	12	68.0723	5.6726	
Total	19	274.9645		

* Transformation of data: $X' = \log (X+1)$

** Significant at 5% and 1% levels.

APPENDIX TABLE 11. The changes in pH of the whey preserved with 0-0.4% sodium sulfite, during storage at 70-72°F.

Sodium sulfite %	pH			
	Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	4.80	5.60	6.70
0.10	"	4.80	4.90	4.85
0.20	"	4.90	4.95	4.90
0.30	"	5.00	4.95	4.80
0.40	"	5.00	4.90	4.90

APPENDIX TABLE 12. The changes in pH of the whey preserved with 0.4% sodium sulfite at varying pH, during storage at 70-72°F.

Sodium sulfite %	pH			
	Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	3.45	4.00	4.50
0.40	3.50	3.50	3.60	3.60
"	4.40	4.00	4.20	4.20
"	5.00	4.90	4.90	5.00
"	6.00	5.70	5.70	6.50
"	7.00	5.90	5.40	6.90

APPENDIX TABLE 13. The effect of sodium sulfite concentration on lactose content of the whey during storage at 70-72°F.

Sodium Sulfite %	Initial pH	Lactose %			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.0	5.76	5.22	3.42	<0.50
0.10	"	"	5.25	4.72	1.60
0.20	"	"	5.50	5.15	4.42
0.30	"	"	5.52	5.30	4.70
0.40	"	"	5.64	5.50	5.20

APPENDIX TABLE 14. Analysis of variance of Appendix Table 13: The effect of sodium sulfite concentration on lactose content of the whey.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	10.4821	2.6205	2.4263 = F_T n.s.
Blocks	3	19.6262	6.5420	6.0573 = F_B *
Error	12	12.9602	1.0800	
Total	19	43.0685		

* Significant at 5% and 1% levels.

APPENDIX TABLE 15. The effect of pH on lactose content of the whey preserved with 0.4% sodium sulfite, during storage at 70-72°F.

Sodium Sulfite %	Initial pH	Lactose %			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	5.76	5.34	4.12	1.12
0.40	3.50	"	5.78	5.69	5.69
"	4.40	"	5.69	5.64	5.69
"	5.00	"	5.52	5.30	4.70
"	6.00	"	4.76	2.00	0.50
"	7.00	"	4.40	0.50	0.50

APPENDIX TABLE 16. Analysis of variance of the Appendix Table 15: The effect of pH on lactose content of the whey preserved with 0.4% sodium sulfite.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	37.3212	9.3303	4.2773 = F_T*
Blocks	3	21.8354	7.2784	3.3367 = F_B*
Error	12	26.1760	2.1813	
Total	19	85.3326		

* Significant at 5% level.

APPENDIX TABLE 17. The effect of sodium metabisulfite concentration on the viable bacterial cells of the whey preserved at its original pH 5.0, during storage at 70-72°F (Trial 1).

Sodium Metabisulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	1.7×10^5	18.2×10^6	*8.8×10^8	*6.0×10^6
0.05	"	"	1.0×10^2	-	-
0.10	"	"	3.2×10^3	2.2×10^5	8.0×10^6
0.15	"	"	5.0×10^3	0.0	*0.0
0.20	"	"	1.1×10^1	-	0.0
0.30	"	"	0.0	0.0	0.0

* Moldy

APPENDIX TABLE 18. The effect of sodium metabisulfite concentration on the viable bacterial cells of the whey preserved at its original pH 5.0 during storage at 70-72°F. (Trial 2).

Sodium Metabisulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	2.5×10^5	16.4×10^7	24.6×10^8	*1.5×10^8
0.05	"	"	14.2×10^3	*	*
0.10	"	"	6.2×10^3	*	*
0.15	"	"	1.6×10^3	0.0	0.0
0.20	"	"	2.6×10^1	0.0	0.0
0.30	"	"	0.0	0.0	0.0

* Moldy

APPENDIX TABLE 19. Analysis of variance of*Table 6: The effect of sodium metabisulfite concentration on viable bacterial cells of the whey.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	5	108.7000	21.7399	7.2664 = F_T **
Blocks	3	28.1464	9.3821	3.1359 = F_B N.S.
Error	15	44.8774	2.9918	
Total	23	181.7238		

* Transformation of data: X' = log (X+1)

** Significant at 5% and 1% levels.

APPENDIX TABLE 20. The effect of 0.15% sodium metabisulfite on the viable bacterial cells of the whey preserved at pH ranging from 3.5-7.0, during storage at 70-72°F (Trial 1).

Sodium Metabisulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	1.7×10^5	8.2×10^7	1.6×10^8	2.8×10^9
0.15	3.50	"	0.0	0.0	-
"	4.40	"	0.0	0.0	0.0
"	5.00	"	2.1×10^3	0.0	*
"	6.00	"	9.8×10^5	*2.0×10^6	*
"	7.00	"	9.4×10^6	*3.0×10^6	*4.0×10^8

* Moldy

APPENDIX TABLE 21. The effect of 0.15% sodium metabisulfite on the viable bacterial cells of the whey preserved at pH ranging from 3.5-7.0, during storage 70-72°F. (Trial 2).

Sodium Metabisulfite %	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	2.5×10^5	6.2×10^6	20.2×10^6	*19.6×10^6
0.15	3.50	"	0.0	0.0	0.0
"	4.40	"	0.0	0.0	0.0
"	5.00	"	4.5×10^3	0.0	0.0
"	6.00	"	7.0×10^6	9.2×10^7	16.2×10^8
"	7.00	"	4.0×10^6	11.1×10^7	*9.4×10^9

* Moldy

APPENDIX TABLE 22. Analysis of variance of *Table 7: The effect of pH on the viable bacterial cells of the whey preserved with 0.15% sodium metabisulfite.

Source of variation	d.f.	S.S.	M.S.	F
Treatment	4	156.7964	39.1991	7.0193 = F_T **
Blocks	3	14.8051	4.9350	0.8837 = F_B n.s.
Error	12	67.0134	5.5844	
Total	19	238.6149		

* Transformation of data: $X' = \log (X+1)$

** Significant at 5% and 1%.

APPENDIX TABLE 23. The changes in pH of the whey preserved with 0-0.30% sodium metabisulfite, during storage at 70-72°F.

Sodium Meta-bisulfite %	pH			
	Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	4.80	5.60	6.70
0.05	"	4.90	4.80	5.00
0.10	"	4.90	4.80	4.70
0.15	"	4.90	4.80	4.80
0.20	"	4.90	4.80	4.75
0.30	"	4.80	4.80	4.60

APPENDIX TABLE 24. The changes in pH of the whey preserved with 0.15% sodium meta-bisulfite at pH ranging from 3.5-7.0, during storage at 70-72°F.

Sodium Meta-bisulfite %	pH			
	Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	3.45	4.00	4.50
0.15	3.50	3.50	3.45	3.40
"	4.40	4.20	4.10	4.00
"	5.00	4.90	4.80	4.70
"	6.00	5.90	5.30	7.60
"	7.00	6.10	5.70	8.00

APPENDIX TABLE 25. The changes in lactose content of the whey preserved with 0-0.30% sodium metabisulfite, during storage at 70-72°F.

Sodium Meta-bisulfite %	Initial pH	Lactose %			
		Initial	After 3 days	After 10 days	After 21 days
0.00	5.00	5.76	5.22	3.42	0.50
0.05	"	"	5.34	4.72	1.80
0.10	"	"	5.56	5.10	3.20
0.15	"	"	5.39	5.34	4.00
0.20	"	"	5.60	5.47	5.03
0.30	"	"	5.69	5.56	5.43

APPENDIX TABLE 26. Analysis of variance of the Appendix Table 25: The changes in lactose content of the whey preserved with 0 - 0.30% sodium metabisulfite.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	5	11.1745	2.2349	2.5121 = F_T n.s.
Blocks	3	22.7819	7.5939	8.5359 = F_B *
Error	15	13.3447	0.8896	
Total	23	47.3012		

* Significant at 5% and 1% levels.

APPENDIX TABLE 27. The effect of pH on lactose content of the whey preserved with 0.15% sodium metabisulfite, during storage at 70-72°F.

Sodium Meta-bisulfite %	Initial pH	Lactose %			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	5.76	5.34	4.12	1.12
0.15	3.50	"	5.76	5.69	5.52
"	4.40	"	5.69	5.60	5.30
"	5.00	"	5.30	5.42	4.00
"	6.00	"	4.00	0.50	0.00
"	7.00	"	4.90	0.50	0.00

APPENDIX TABLE 28. Analysis of variance of the Appendix Table 27: The effect of pH on lactose content of the whey preserved with 0.15% sodium metabisulfite.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	41.4965	10.3741	4.3719 = F_T *
Blocks	3	27.6157	9.2052	3.8793 = F_B *
Error	12	28.4743	2.3728	
Total	19	97.5865		

* Significant at 5% level.

APPENDIX TABLE 29. The effect of 250 p.p.m. hydrogen peroxide on the viable bacterial cells of the whey preserved at pH ranging from 3.5-7.0, during storage at 70-72°F. (Trial 1).

Hydrogen Peroxide	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	4.0×10^4	11.1×10^6	9.2×10^8	23.5×10^8
250 p.p.m.	3.50	"	0.0	0.0	0.0
"	4.40	"	1.8×10^2	0.0	0.0
"	5.00	"	8.2×10^4	4.6×10^6	1.4×10^7
"	6.00	"	18.2×10^6	6.2×10^6	12.4×10^6
"	7.00	"	8.4×10^7	1.5×10^7	1.2×10^7

APPENDIX TABLE 30. The effect of 250 p.p.m. hydrogen peroxide on the viable bacterial cells of the whey preserved at pH ranging from 3.5-7.0, during storage at 70-72°F (Trial 2).

Hydrogen Peroxide	Initial pH	Viable bacterial cells per ml			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	3.6×10^4	9.6×10^5	4.1×10^7	5.1×10^7
250 p.p.m.	3.50	"	0.0	0.0	0.0
"	4.40	"	8.4×10^1	0.0	0.0
"	5.00	"	1.3×10^5	1.6×10^6	3.8×10^5
"	6.00	"	2.2×10^6	3.8×10^6	1.9×10^4
"	7.00	"	17.8×10^7	6.8×10^6	6.2×10^6

APPENDIX TABLE 31. Analysis of variance of *Table 8: The effect of pH on the viable bacterial cells of the whey preserved with 250 p.p.m. hydrogen peroxide.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	150.8982	37.7245	7.6828 = F_T **
Blocks	3	1.1184	0.3728	0.0759 = F_B n.s.
Error	12	58.9226	4.9102	
Total	19	210.9392		

* Transformation of data: $X^1 = \log (X+1)$

** Significant at 5% and 1% levels.

APPENDIX TABLE 32. The changes in pH of the whey preserved with 250 p.p.m. hydrogen peroxide, during storage at 70-72°F.

Hydrogen Peroxide	pH			
	Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	3.45	4.00	4.50
250 p.p.m.	3.50	3.50	3.45	3.40
"	4.40	4.10	4.00	4.30
"	5.00	4.70	5.40	6.70
"	6.00	5.00	4.20	4.75
"	7.00	5.00	4.40	4.80

APPENDIX TABLE 33. The effect of pH on lactose content of the whey preserved with 250 p.p.m. hydrogen peroxide, during storage at 70-72°F.

Hydrogen Peroxide	Initial pH	Lactose Percentage			
		Initial	After 3 days	After 10 days	After 21 days
0.00	3.50	5.76	5.34	4.12	1.12
250 p.p.m.	3.50	"	5.73	5.69	5.69
"	4.40	"	5.69	5.64	5.69
"	5.00	"	5.43	3.76	1.60
"	6.00	"	5.20	2.20	0.50
"	7.00	"	5.10	0.92	0.00

APPENDIX TABLE 34. Analysis of variance of the Appendix Table 33: The effect of pH on lactose content of the whey preserved with 250 p.p.m. hydrogen peroxide.

Source of variation	d.f.	S.S.	M.S.	F
Treatments	4	27.2297	6.8074	3.3504 = F_T*
Blocks	3	33.7033	11.2344	5.5292 = F_B*
Error	12	24.3818	2.0318	
Total	19	85.3148		

* Significant at 5% level.