THE RELATIONSHIP OF CATALASE ACTIVITY IN RAW MILK TO QUALITY OF THE PROCESSED PRODUCT

By

EDGAR DEAN SMITH, B. S.

A THESIS

IN

FOOD TECHNOLOGY

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

MASTER OF SCIENCE

Approved

Accepted

December, 1972

ACKNOWLEDGMENTS

The author wishes to express his deep and sincere appreciation to Dr. R. M. Miller for his guidance, patience, and suggestions throughout the course of this graduate program.

The author also wishes to thank Dr. M. L. Peeples for his interest in the problem, encouragement, and his suggestions concerning laboratory techniques, and composition and presentation of the data.

Grateful acknowledgment is due to Dr. L. E. Blakely for his assistance, advice, and concern; to Dr. L. C. Kuhnley for his advice and effort in reading this thesis; to Mr. W. B. Gilmore for his cooperation in allowing me the use of the University Creamery facilities; to Bell Dairy Products, Inc. for giving me several milk samples with which to work; and to the Department of Food Technology at Texas Tech University for the financial support that made this project possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

A problem of major concern to the dairy industry today is the shelf life of market milk. Although many projects have been devoted to finding a reliable method for assessing the quality of milk, to date, a satisfactory test has not been developed for differentiating between raw milk which may have a potentially unsatisfactory shelf life in the processed state, and raw milk which may have excellent keeping quality after processing.

The results of recent work in Australia suggest the possibility that catalase activity of raw milk may be associated with gram-negative bacterial growth which in turn has been shown to be associated with short shelf life of dairy products.

Accordingly, the objective of this research was to modify the above-mentioned catalase test so that it may serve as a procedure for indicating the shelf life potential of raw milk after pasteurization.

CHAPTER II

REVIEW OF LITERATURE

Shelf Life of Market Milk

Barnard (11, 12) has noted that today's consumer determines the acceptance of fluid milk based upon flavor and shelf life of this food product. Ideally market milk should possess no off-flavor if consumed within a reasonable period of time after processing. However, the abuse given this product with regard to storage temperatures at both retail outlets and the consumer's home may result in early off-flavor development (36, 51).

For example, one factor which may result in market milk having a short shelf life is that associated with temperature variations of self-service dairy cabinets. Based on a survey (54) of self-service dairy cabinets, researchers found that the temperatures varied from 32-51 F. To determine the effect of storage cabinet temperature variations upon the shelf life of homogenized milk, studies were made using samples from four widely separated distributors. Samples were stored at 36, 41, 46, and 51 F, and samples for analysis were taken after 2, 4, 7, and 10 days storage. Milk held at 36 F showed little or no change in flavor or bacterial counts during the ten day storage period. When milk was held at 41 F increases in psychrophilic counts were observed and off-flavors were present after storage at 7 and 10 days. Samples stored at 46 and 51 F maintained acceptable flavor quality for approximately 4 days. Standard plate counts and coliform counts increased steadily, during the

10 day storage period, for samples held at 46 F. Milk stored at 51 F exhibited very rapid bacterial growth, as determined by the standard plate count. Coliform counts on the samples stored at 51 F increased during the first seven days of storage, then decreased sharply by the tenth day. Slight increases in thermopholic counts were noted on samples stored at 51 F.

Several investigators (20, 28, 29, 150) have shown that good quality milk, properly processed, can be kept for a considerable length of time in household refrigerators under laboratory conditions without impairment to its nutritional qualities, flavor score, or bacteriological safety. However, household refrigeration practices vary so greatly that researchers have had a difficult time attempting to duplicate household milk handling conditions.

Overcast (115) and Langlois, et al., (69) found that improper refrigeration of milk was a common occurrence in the average household. At common household refrigeration temperatures there was one chance in eight that milk would have a noticeable off-flavor after 4 days storage. At the end of seven days there was a twenty per cent chance that the product would have an off-flavor (147). Weese, et al., (150) concluded that efforts should be made to educate the consumer toward a better appreciation for proper refrigeration of milk.

Because of the importance of flavor and shelf life of market milk and since shelf life is so ill-defined and hard to control, it is necessary that a long shelf life milk meet certain criteria. Hedrick (51) defined a long shelf life market milk as one which showed no detectable sensory deterioration at the place of utilization for at least two weeks

and which had microbiological numbers no greater than 20,000 per ml (145). In 1953 the average shelf life of whole milk pasteurized by the H.T.S.T. method was about 10 days under normal storage conditions (109), while today milk keeps satisfactorily for about 14 days (16, 20). In this connection, it was learned in a private interview (Brooks, C. E. 1971) that a local dairy concern has taken steps to increase the shelf life of their market milk to 21 days. This firm has suggested to dairy farmers that they keep milk storage temperatures below 40 F. The firm also plans to change from water-cooled receiving tanks which cool milk to 39 F to glycol-cooling equipment in order to lower the temperature in the bulk milk receiving tanks to 32-33 F. The storage vaults would also be glycol-cooled. Such an improvement would allow processed milk to leave the storage vaults at a desired 35 F instead of the present 40 F. Also, this dairy concern has planned to pasteurize at 195 F in order to kill more psychrophiles, and management has emphasized that milk should not remain in the retail store for more than 24 hours. Other measures this dairy firm has taken in order to increase the shelf life of their market milk are (i) using only good quality raw milk (51), (ii) insisting on adequate pasteurization time and temperature (50), (iii) avoiding repasteurization when possible (99), (iv) maintaining constant vigilance on cleaning equipment (50, 132), (v) preventing post-pasteurization contamination (51), (vi) holding and merchandising at 4.4 C or below (33, 37, 51, 54, 99, 136), (vii) re-educating the customer (51) and (viii) strictly controlling routine bacteriological testing (99).

Many dairy scientists suggest that laboratory personnel check samples

which are in retail outlets more closely (50, 99). Grosshopf and Harper (43) found that fluid milk pasteurized aseptically and stored at 4.4 C had a shelf life of 4 weeks. Other researchers (20, 82, 98) found that pasteurized milk could be stored at 40 F for a period of two weeks or more before spoilage occurred. If the storage temperature was raised from 40 to 45 or 50 F all samples were often unpalatable after 4 days (19). If samples which were stored at 40 F were removed from the refrigerator each day, shaken, and allowed to stand at room temperature for one hour, off-flavors usually developed within 10 days (20, 24). However, if the storage temperature is lowered from 40 F to 33 F, the average shelf life of the milk may be extended another 11 to 14 days (16).

Langlois and Rudnick (69) observed the keeping quality of homogenized milk, skim milk, lowfat milk, chocolate milk, half and half, and whipping cream and found that homogenized milk had the poorest keeping quality of this group of dairy products. This was explained by the fact that other products generally received a somewhat higher heat treatment than does homogenized milk. The homogenized milk also had the poorest flavor at time of purchase. Almost 7 per cent of the homogenized milk samples had a flavor score of less than 36 when purchased. The shorter shelf life of the homogenized milk samples was partially explained by the bacterial flora found in this product. Most of the homogenized milk samples that had a shelf life of 7 days or less had total plate counts in excess of 20,000 bacteria per ml and psychrophilic counts in excess of 1,000 bacteria per ml at the end of 7 days. The researchers also found that milk samples which had a shelf life of more than 7 days had the same bacterial numbers, but only 50 per cent of those samples contained greater than 1,000

psychrophilic bacteria per ml. They concluded from these data that total numbers were much less important than psychrophilic numbers when evaluating the potential shelf life of milk products. Disregarding the cause of spoilage, the data showed that except for low fat milks the housewife had a 5 per cent chance of a particular purchase having an off-flavor (69).

Although psychrophilic bacteria surviving pasteurization have been found to result in off-flavor development in market milk, post-pasteurization contamination appears to be the major contributor to the off-flavors in this product (32, 68, 82, 92, 125). Elliker, et al., (34) pointed out that contaminated equipment between the pasteurizer and the final container was the most important source of bacteria in pasteurized milk. Cleaning water, pipe lines, holding tanks, pumps, and valves as well as milk cans and milking machines have all been cited as sources of psychrophilic bacteria in milk (55, 63, 66).

Overcast (115) sampled air from one milk plant using an Anderson air sampler. Twenty-four samples averaged 14 psychrophiles per cubic foot. On a volume basis, this would mean one psychrophile for every half gallon carton. In addition, 45 per cent of the carton stock examined, in a number of trials, contained one or more psychrophilic cells, thus presenting a threat that had been considered negligible in the past (33, 115). In another study (21), the airborne bacteria isolated in milk processing areas were principally bacilli, corynebacteria, micrococci, and gram-negative rods (excluding coliforms). Twenty-five per cent of the isolates were found to be psychrophiles. These were primarily bacilli and gram-negative rods (excluding coliforms). There was no apparent relationship between

airborne microbial populations and the keeping quality of packaged milk.

Post-pasteurization contamination was observed by Maxcy (81), to contribute insignificantly to the total count in freshly pasteurized packaged milk. Such contamination however, contributed most of the bacteria that are capable of causing spoilage during subsequent storage of this product. The study also revealed that 99 per cent of the bacteria contributing to the total plate count in pasteurized packaged milk were of minor significance with regard to spoilage. The gram-negative rods contributed by post-pasteurization contamination, although small in number, were very significant with respect to spoilage.

Shelf Life Prediction Tests

To date, there is not a satisfactory test for predicting the shelf life of pasteurized, packaged market milk (150). Several investigators (5, 14, 15, 22, 54, 68, 97, 109) have attempted to show a correlation between the standard plate count and shelf life of market milk, but all have concluded that no close correlation exists. The claim is made (8, 47, 55) that a wide margin of error appears inevitable in the standard plate count. Another disadvantage of the count is that heat-resistant bacteria which do not significantly affect the keeping quality of milk are included. The standard plate count also requires a considerable amount of equipment, and a 48 hour incubation period is necessary prior to obtaining results (47).

Recently the shelf life of market milk has been best reflected by "Five Day - 45 F Counts" (34, 59, 60, 134, 147). This technique appeared to distinguish between milk samples that have passed through properly

cleaned versus improperly cleaned equipment after pasteurization. The test involved making a standard plate count of the freshly pasteurized product and repeating the determination on the same milk sample after a 5 day holding period at 45 F. The correlation coefficient between bacterial numbers and shelf life of milk has been reported as 0.95 (a perfect correlation is 1.0).

The effect of storage temperatures and times upon the fluctuations in bacterial numbers in commercially pasteurized milk has been studied (147). Watrous et al., (148) noted that bacterial numbers increased as the storage times and temperatures increased. However, the data showed that changes in counts as a function of time and temperature bore no relationship to the count of the original sample. The shelf life of market milk could not be accurately predicted based upon standard plate counts taken immediately after the milk had been pasteurized. The data also revealed that psychrophilic bacteria were not recovered from freshly pasteurized milk after 5 days storage at 4.4 C. After 10 days incubation at 7.2 C surviving thermodurics began growing, emphasizing the need for low storage temperatures.

Workers such as Egdel and Bird (32) have indicated that standard plate counts taken at various processing stages in a commercial operation are not good indicators of post-pasteurization contamination. Another study (54) indicated that the incubation temperature and time currently recommended for the standard plate count, while adequate for the enumeration of bacteria in raw milk, may not be satisfactory for the enumeration of bacteria in pasteurized milk because these spoilage bacteria are present in such small numbers.

Some early workers believed that the coliform counts, after preliminary incubation, were a reliable keeping quality indicator test (14, 16, 59, 96, 146). Punch et al., (122) and Witter (153) stated that to date, a test for accurately predicting the keeping quality of milk does not exist. They added that such a test, in order to be effective, must account for the level of psychrophilic contamination, increases in psychrophilic growth with storage time and temperature, and bacterial activity in producing deleterious effects. Several other researchers disagreed with Witter and have reported that initial psychrophilic counts did not serve as a basis upon which shelf life predictions may be made (14, 20). However, it was found that immediately after pasteurization milk samples with populations of 10 or less psychrophiles per ml had long shelf lives (18).

Witter (153) suggested that the psychrophilic count has been of little value for predicting keeping quality because a low level of active bacterial contamination may result in a shorter shelf life than that resulting from a relatively large number of biochemically inert psychrophiles.

Two general types of procedures have been employed (64) for estimating shelf life of pasteurized milk. One test encouraged the growth of psychrophilic contaminants in refrigerated storage before testing; another, applied to fresh samples, employed the use of inhibitory chemicals to repress gram-positive bacteria.

A promising test employing inhibitory chemicals is known as the CVT test (crystal violet-tetrazolium chloride test). The test was developed by Olson (101, 102, 103, 107) after he observed that

microorganisms which survived milk pasteurization were generally gram-positive types, while the organisms usually responsible for spoilage in pasteurized milk were gram-negative. These gram-negative micro-organisms generally grew well, and developed colonies on agar containing 2,3,5 triphenyl tetrazolium chloride while the gram-positive types developed little or no color (31, 106). Olson utilized crystal violet to inhibit gram-positive organisms. Red colonies on this crystal violet-triphenyl tetrazolium chloride agar were usually associated with post-pasteurization contamination. Since the medium detected coliforms and other gram-negative organisms the test was a good method for locating post-pasteurization contamination (103, 108). Olson (106, 107, 108) plated pasteurized milk on the CVT medium immediately after pasteurization and after storing the milk for 5 days at 45 F. This method restricted the growth of organisms normally surviving pasteurizatio while permitting the growth of gram-negative post-pasteurization contaminants which were common causes of spoilage (103).

Olson and Chaudhari (106) in analyzing twenty tests designed for predicting shelf life found no meaningful correlation between any of the tests and milk shelf life at 45 F. The best tests appeared to be the initial CVT count, initial psychrophilic count, CVT count after 24 hours storage at 55 F, and after 24 hours storage at 60 F. There were no significant correlations between any of the CVT counts and psychrophili counts except where the latter were rather high. The CVT and initial counts proved to be better than other tests studied when attempting to predict market milk shelf life. The CVT test was preferred over psychrophilic counts because the test required a shorter time to obtain

results. Olson (101) also pointed out that the type of organisms present were more nearly related to shelf life than were total bacterial numbers. An initial count of less than one per ml of a vigorous spoilage organism can cause short shelf life of market milk (42). On the other hand, evidence of contamination as indicated by the CVT test did not necessarily assure that milk would spoil rapidly (103).

Edwards (31) indicated that there was not a correlation between milk degradation rates and CVT counts. He also concluded that the CVT test was an excellent indicator of post-pasteurization contamination but was not an adequate method for predicting market milk shelf life.

Various other methods for predicting shelf life of market milk have been attempted (30, 31, 59, 83, 84, 87); of these, the clot-on-boiling (C. O. B.) test was considered the most accurate in Europe, although it chiefly reflected susceptibility of the milk to souring. In this test, portions of the sample were maintained at 18 C followed by submerging in boiling water and examining for clotting; this continued until clotting occurred. This method proved to be too cumbersome and time consuming for routine testing.

The dye reduction test was also used to assess probable keeping quality of pasteurized milk in Europe. However, the test was not considered a reliable prediction test (64).

Psychrophilic Bacteria

Although the major detriment to good keeping quality in pasteurized milk today appears to be associated with psychrophilic bacteria (34), and although there is no all inclusive definition of psychrophiles which

is accepted by everyone, these bacteria have been defined (153) according to (i) optimum growth temperature, (ii) ability to grow at refrigeration temperatures, (iii) the method of enumeration, and (iv) criteria which are independent of temperature, e.g. defects produced. The dairy industry (115) considers psychrophilic bacteria as those organisms that grow in milk or its products at refrigeration temperatures (usually less than 10 C) at such a rate as to produce off-flavors and odors in a relatively short period of time, and which will eventually render the product unpalatable (46).

Some generalized characteristics of psychrophilic bacteria are that (i) they do not survive pasteurization (115, 153), (ii) they are predominantly gram-negative rods (111), and (iii) they are predominantly catalase positive (17, 84, 153), and (iv) they are asporogenic, resistant to penicillin, non-acid forming, and resistant to toxicity of basic dyes (100, 101, 102, 103). Generally these organisms are of soil and water origin (15).

Overcast (115) noted that as psychrophiles grew in milk rather subtle changes in the milk took place in the milk during the early growth period. From a physical standpoint, noticeable changes due to psychrophiles were not evident immediately in the milk, but tastewise, one might first be conscious of a lack of freshness followed by a change to slightly stale due to metabolism of psychrophilic bacteria. Subsequently, more objectionable flavors developed including rancid, fruity, bitter, putrid, cheesy, skunky, or fishy (115). Other defects that may develop are discoloration, ropiness, potato odor, and aroma loss (153). Only a small percentage of the psychrophiles common

to the dairy industry are acid producers and these generally produce other off-flavors before acid-producing organisms can dominate the population.

Several investigators (1, 16, 24, 32, 153) isolated and characterized psychrophilic organisms capable of dominating the populations in commercially pasteurized samples of milk after storage at 4 C. Most of the isolates were gram-negative bacilli and belonged to either the: a) Achromobacter, b) Alcaligenes, c) Pseudomonas, d) Flavobacterium, or e) the coliform group. Pseudomonas accounted for seventy per cent of the isolates found in milk. Representatives of this genus which were of particular importance to the milk industry were Pseudomonas fragi and Pseudomonas fluorescens (153). These bacteria often contaminated pasteurized milk in very small numbers. Many times less than 10 per ml were found in fresh pasteurized milk (23). Their source was occasionally potable water used for rinsing cleaned equipment. A small number of psychrophiles per quart of milk from such sources could multiply sufficiently to cause spoilage if the refrigeration temperature was too high. A common spoilage defect observed in pasteurized milk was fruity off-flavor, caused by Pseudomonas fragi (23).

Psychrophilic bacteria such as Pseudomonas fluorescens and particularly Pseudomonas putrefaciens were associated with partial proteolysis of casein which may have caused undesirable flavors in milk (51, 60, 120, 133). In a study (146) of the proteolytic activity of psychrophilic bacteria isolated from refrigerated, pasteurized milk, 75 per cent of the psychrophiles studied grew over a wide range of temperatures. With the exception of one, all organisms had a short lag

phase even at low temperatures. No relationship between the psychrophilic bacteria numbers and proteolytic activity was evident.

To delineate more specifically the action of some dairy psychrophiles on milk proteins, the caseins and whey proteins were obtained after psychrophilic growth and separated electrophoretically. Overcast (115) found that a decrease in the total protein content occurred. He suggested that a more detailed study of the changes in milk proteins might indicate a close relationship to off-flavors produced by the organisms.

Most of the psychrophilic bacteria that caused off-flavors in pasteurized milk have been found to be proteolytic in nature, with practically no ability to attack carbohydrates (44). Storage of milk at low temperatures was selective against those bacteria which normally produced acid from lactose, and selective for the psychrophiles which attacked fats and proteins (58, 128, 138, 146). The activity of proteolytic enzymes (both extracellular and endocellular) increased with increasing temperatures (118).

Two psychrophilic bacteria, Pseudomonas fluorescens, and particularly Pseudomonas fragi, associated with rancidity in market milk (146), liberated free fatty acids which cause a pungent flavor and odor to develop in a milk product during refrigerated storage (57, 110). Small inoculums of certain psychrophilic bacteria produced rancid off-flavors and increased the free fatty acid content three to five fold in milk within 48 hours at 4 C (67). Of twenty-five lipolytic psychrophiles isolated from pasteurized milk and inoculated into sterile milk, ten isolates produced rancidity within 4 days, and fifteen produced

rancidity within 8 days.

The ability of some psychrophiles, particularly some of those of the Pseudomonas species, to attack milk fat could have serious implications since their lipase enzyme(s) have been shown to be resistant to pasteurization temperatures (120). This may be the reason that in some cases microbial lipases appear to be active in pasteurized products where lipolytic bacteria had grown prior to pasteurization (120).

Experimentation (93, 94, 95) with the lipase(s) of Pseudomonas fragi demonstrated that the enzyme(s) was preferentially produced by the microorganisms when grown at 30 C or higher. The optimum temperature and pH for activity of the lipase(s) was found to be 40 C and 7.0 to 7.2, respectively. A heat treatment of 99 C for twenty minutes was required to completely inactivate this enzyme. In this connection, a lipase was discovered from psychrophilic bacteria which resisted boiling for one hour without elimination of its activity (50).

The activities of 23 Pseudomonas species were studied (127) to determine their effects upon the flavor of pasteurized milk during storage. The increase in free fatty acid content, as measured by the acid degree value procedure (39), and flavor changes were noted after 2, 4, 8, and 14 days storage at 4 C. The heat resistant lipase(s) from seven of these cultures produced a slight rancid flavor in pasteurized milk on the second day of storage. Fourteen of these cultures produced rancidity in milk within eight days, and the lipase(s) from all 23 cultures caused rancid flavor to develop within 14 days. The increases in free fat acidity ranged from 0.96 to 2.25 acid degree units. Off-flavor was detectable in two samples where the acid degree

value was only 0.97.

Populations of psychrophilic bacteria associated with detectable off-flavor changes in milk varied among a) genera, b) species within a genus, c) type of organism, d) storage, and e) availability of oxygen (45, 122). Five hundred thousand to twenty million organisms per ml of milk were present before these defects were noticed organoleptically (42, 45, 62, 110, 122, 123).

The generation time of bacteria varied with species and storage temperature (46). For example several species were known to double in number when held at 41 F for 5 hours. At this rate, one psychrophile could result in a population of more than one million per ml in 3.5 days, or milk with an initial count of 500 psychrophiles per ml could have a count almost three million per ml in two days.

Pseudomonas fragi had (115) a generation time of 5.5 hours at 5 C. This bacteria had a rather short lag phase at 5 C and when pasteurized milk was inoculated with as few as 500 cells per ml, counts in the millions per ml resulted after two days storage at the above temperature. Another common dairy psychrophile, Pseudomonas fluorescens had a generation time of 7.2 hours at 5 C; but, even with a longer generation time than Pseudomonas fragi, pasteurized milk inoculated with small numbers of this bacteria had plate counts beyond the acceptable limits after three days.

Overcast (115) cited Olson's (103) calculations ascertaining the theoretical shelf life of a quart of milk containing only one psychrophilic bacterium and stored at 7 C. According to these calculations, a single organism with a generation time of six hours in a quart of milk could multiply to one million per ml in eight days or 73 million

in 9 days. Consequently, a single organism gaining entrance into a carton of milk could result in large bacterial populations. However, Overcast (114) observed that although a noticeable flavor defect usually required large numbers of organisms, subtle changes took place such as "lack of freshness" or "stale quality" even with a few bacteria per ml of milk.

In a study (123) of the action of four lipolytic psychrophilic bacterial cultures on milk, the most commonly detected off flavor was "unclean." Organoleptically detectable changes were not measurable by chemical analysis of fat (acid degree value) or protein hydrolysis. A significant increase in acid degree values was not observed until several hours after an initial change in flavor was noted. In their organoleptic analysis of the milk samples, the judges noted a flavor defect other than unclean occasionally. In such samples the sequential flavor change usually proceeded from astringent to unclean to bitter, fruity, or putrid. The acid degree values correlated with organoleptic evaluations in that normal milk had an acid degree value of approximately 0.5 while hydrolytic rancidity was first detected organoleptically at an acid degree value of 1.5. The milk was considered extremely rancid at an acid degree value of 2.5.

Weber (149) found that pasteurized milk with a high initial psychrophilic count before pasteurization had lower keeping quality than milk with a low psychrophilic count prior to pasteurization. However, Overcast (113) while studying the effect of psychrophilic growth in raw milk upon the growth of psychrophilic bacteria introduced into the milk after pasteurization, noted that excessive psychrophilic

growth in raw milk had a stimulatory effect upon the ability of a psychrophilic isolate to initiate growth after the milk had been pasteurized and stored for one or two days. The effect became slightly but insignificantly inhibitory on the ability of the psychrophilic isolate to grow by the end of 3 to 4 days. These same conditions tended to inhibit the growth of Pseudomonas fluorescens in pasteurized milk after 4 days, although the effect was not statistically significant. Another experiment (78), indicated that psychrophilic bacteria grew faster in pasteurized milk which had a high standard plate count before pasteurization than in pasteurized milk which had a low standard plate count prior to pasteurization.

A considerable amount of work has been performed on the psychrophilic microorganisms in raw milk (4, 55, 61, 77, 78, 79, 113, 121, 135, 139), and their fate during subsequent storage in the pasteurized milk (6, 7, 29, 49, 53, 54, 70, 76, 124, 128, 129). The commonly encountered psychrophiles in raw milk that may cause off-flavor development in pasteurized milk were destroyed by proper pasteurization (46, 82, 113, 115, 153). However, other data (27, 35, 41, 43, 48, 67, 104, 126, 144) indicated the possibility that certain psychrophilic bacteria present in raw milk were able to withstand various pasteurization processes.

In one study (43) psychrophilic spore-formers were isolated from about 25 per cent of the producer's milk supplies tested. Initial results suggested that all of the psychrophilic spore-formers would survive ultra high temperature (U. H. T.) pasteurization. Watrous et al. (147) suggested that spore-formers were associated with the ultimate spoilage of milk even if protected from post-pasteurization

contamination.

One writer (89) suggested that "heat shock" played an important role in the post-pasteurization activity of the germinated psychrophilic spores. Under milk handling practices, "heat shock" may have been significant in situations where raw milk contaminated with spores was subjected to conventional heat treatments which activated the spore and created environmental conditions in the milk favorable for spore germination and consequential vegetative cell growth. If such milk were stored improperly, the investigators surmised that spoilage would occur (89, 137).

Dabbah et al., (27) isolated an unidentified Pseudomonas species from milk heated to 55 C for 30 minutes and stored at 4 C for 48 to 72 hours. The authors contended that the Pseudomonas species, such as the one isolated, may have been heat shocked by the heat treatment and subsequently were capable of contributing to the spoilage of pasteurized milk as were post-pasteurization contaminants. Other workers supported this contention (15, 32, 36, 137, 142). Dabbah, et al., (26) wrote that injury to bacteria from heat shock treatments appeared to alter their subsequent growth response. Therefore, it is difficult to differentiate among a) the kill that occurs during pasteurization, b) thermal injury resulting in delayed growth of the bacteria, and c) subsequent multiplication. Pasteurization processes often involved a heat treatment of 76 C (168.8 F) for 16 seconds (143). Researchers (15, 33, 37, 54, 65, 140, 142) differed with respect to the reported number of organisms surviving this type of pasteurization and subsequently growing in refrigerated milk samples. Watrous et al., (148) suggested

that quality programs advocated by various milk plants have selective action with respect to survival of organisms capable of growing at low temperatures because of the pasteurization methods utilized.

Since the initial bacterial counts obtained on milk immediately after pasteurization may have been affected by so many factors, e.g., type of bacteria present, extent of injury during pasteurization at varying temperatures and times, and variations in methods used for enumerating, then it might have been expected that workers have had difficulty in correlating bacterial counts of milk, in the initial stages of storage, with shelf life of the product (15, 26, 33, 37, 42, 54, 82, 142, 143, 148).

It has been suggested (118) that one or more of the following may be reasons for the development of flavor defects: (i) end products of microbial metabolism in raw milk may become apparent in the pasteurized product; (ii) constituents of large numbers of heat inactivated and lysed cells may impart off-flavors to the pasteurized milk; (iii) heat stable microbial enzymes produced in raw milk may remain active after pasteurization and cause changes in some constituents of the pasteurized milk during storage; and (iv) presence of thermoduric psychrotrophs and their growth in pasteurized milk during prolonged storage.

Milkstone

One of the major considerations influencing market milk shelf life is that of milkstone accumulation on processing equipment (86).

Milkstone has as its origin a deposit of milkfilm which forms on

metal heat-transfer surfaces due to the precipitating action of heat (63, 73). The dairy industry considers milkstone as the product resulting from complex reactions involving milk film, chemical constituents of water (primarily calcium and magnesium salts), and alkaline detergent cleaners. Thus, milkstone is a heterogeneous mixture of organic and inorganic substances that adheres tenaciously to milk heating surfaces (71). The milkstone problem is especially associated with high-temperature-short-time pasteurization (H.T.S.T.) equipment because such large volumes of milk are heated to high temperatures in relatively confined spaces (71).

Milkstone should be removed from heating surfaces for several reasons (71, 86) namely, a) milkstone acts as an insulator when deposited in several layers -- thus reducing heat transfer, b) milkstone serves as a source of off-flavors and odors, c) it is unsightly, d) accumulations are difficult to remove, and e) milkstone has been reported as the most common cause of high termoduric counts in pasteurized milk. Unless removed, milkstone serves as a focus for seeding the milk with bacterial contaminants (13, 35).

Johns (63) concluded from his studies that, in spite of increased knowledge and improved detergent formulation, milkstone formation continues to present a problem, especially in hard water regions. He recommended the use of non-ionic wetting systems and a iodophor sanitizer on equipment before every processing operation to prevent milkstone buildup. Leggatt added (72) that it has been clearly established that if all milkstone is not removed, chemical sterilization of equipment will be a failure.

Accordingly, Maxcy (81, 85), after attempting to sterilize milkstone-contaminated equipment, observed that the microflora of pasteurized, packaged milk was similar to the microflora of the milkstone-contaminated equipment, through which the milk had been processed except that a) bacilli occurred more frequently in the pasteurized, packaged milk than on the milkstone contaminated equipment, and b) there was more frequent occurrence of gram-negative rods on the equipment than in the milk.

In order to determine the extent and nature of the microorganisms which contaminated products during processing, researchers have (13, 80, 81, 105, 141) investigated automated circulation techniques (cleaned-in-place) for cleaning dairy equipment. When automated dairy equipment was properly C.I.P. cleaned, the microorganisms remaining for growth and subsequent contamination of milk were relatively few but represented a heterogenous lot. The results also indicated that although gram-negative rods accounted for only a small percentage of the numbers of organisms associated with the equipment, closed systems and extensive mechanical handling of milk favored the growth of these gram-negative bacteria.

Catalase

One investigator (38) stated that the natural enzymes in milk seem to have little importance with reference to the keeping quality of dairy products. However, measurement of the catalase enzyme activity in butter has been used as a keeping quality test, based on the premise that psychrophilic bacteria which spoil butter produced large amounts

of this enzyme (153).

Accordingly, Loane (74) investigated the catalase activity in raw milk. Hydrogen peroxide was added to raw milk samples to a concentration of 0.04 per cent, after which the samples were immediately incubated, and later subjected to a residual hydrogen peroxide test involving the addition of forty per cent potassium iodide (116). Lack of color development indicated a reduction in hydrogen peroxide concentration and hence an appreciable degree of catalase activity. The raw milk samples were also scored organoleptically. Statistical analysis of the results indicated that there was a positive correlation between the residual hydrogen peroxide test and arbitrary bacteriological standards for a satisfactory raw milk.

A positive correlation was also found between the residual hydrogen peroxide test and organoleptic scores of the raw milk provided the milk was tested and organoleptically scored on the same day. Although the test did not predict keeping quality, it did very significantly reflect microbial activity at the time of testing.

There are two sources of catalase in cow's milk -- the mammary gland and bacteria (52, 110, 131). Catalase activity has been noted in milk drawn aseptically from healthy cows (91), with relative activity varying among breeds and the fraction of the milking. In one study, the first portion was poor, and the last portion was rich in this enzyme (117). Furthermore, high leucocyte counts have been associated with increased catalase contents (52, 56, 57, 68, 88, 110, 117, 152). However, even though catalase activity may vary according to these factors, most of the catalase activity in market milk is of saprophytic, psychrophilic

bacterial origin (68, 74, 91, 110, 117). Researchers have reported that most of the true lactic acid bacteria present in milk did not produce catalase (112).

According to the literature (92), the presence of more than 2 x 10^5 bacteria per ml of milk of active catalase producing bacteria are needed to contribute significantly to the catalase content of the sample. The most active producing cultures of Bacillus subtilis significantly increased catalase scores of one experiment only when the bacterial populations were 2 x 10^5 bacteria per ml or higher. Since the legal standard in the United States for commingled grade A raw milk to be pasteurized is not more than 3 x 10^5 bacteria per ml, and for manufacturing grade milk not more than 1 x 10^6 bacteria per ml, the contribution of bacteria to the catalase scores of milk is not likely to be significant unless the organisms consist entirely of such active catalase producing bacteria (92) as those gram-negative microorganisms normally associated with off-flavor development in refrigerated milk (74).

Environmental factors also affect catalase activity (9). In one study (10), the optimum temperature and pH for enzyme activity was found to be 22 C and 5.6 to 10.7 respectively. Another investigator (68) stated that catalase exhibits optimum activity at pH 7 and at a temperature range of 0 to 10 C.

When heated above 45 C, catalase lost its activity rapidly and became inactive at 65 C. Speck (137) noted that the catalase enzyme was inactivated by ultra-high-temperature pasteurization temperatures. However, partial reactivation of catalase enzymes has been found in

properly pasteurized milk (124).

There are many factors to be considered when developing a test utilizing a free-radical former such as hydrogen peroxide (90, 130) and an enzyme system such as catalase in milk. In Loane's (74) test, hydrogen peroxide was added to the milk to provide a substrate for the milk catalase (92) even though hydrogen peroxide was somewhat bactericidal (9). There are conflicts in reports concerning the bactericidal effectiveness of hydrogen peroxide. Babel and Hammer (9) noted that catalase producing organisms were readily destroyed by hydrogen peroxide, but another investigator (130) revealed that hydrogen peroxide was toxic to organisms not possessing catalase producing ability. Babel et al., (9) reported that treatment of milk with 0.2 per cent hydrogen peroxide, followed by the addition of catalase to remove any residual hydrogen peroxide, compared favorably with pasteurization as a means of bacterial destruction. Anaerobic spore-forming organisms were eliminated by the hydrogen peroxide treatment, but not by pasteurization.

One scientist (23) found that the time required to destroy various organisms with 500 p.p.m. (by weight) of hydrogen peroxide at 96.6 F ranged from 40 minutes with Alcaligenes viscosus to 1,080 minutes with a Bacillus species. Catalase-producing organisms in milk were destroyed readily by the above treatment. At ambient or lower temperatures, decomposition of aqueous solutions of hydrogen peroxide was slight, and bactericidal effectiveness was low. At temperatures higher than 96.6 F, bactericidal efficiency increased and the rate of hydrogen peroxide decomposition was accelerated.

The catalase in milk greatly accelerated the decomposition of hydrogen peroxide, with the liberation of molecular oxygen and water (68, 92). In fact, the results of one experiment indicated that there was rapid decomposition of hydrogen peroxide in raw milk at 37.8 and 48.9 C during the first six minutes of incubation due to the activity of catalase and lactoperoxidase (3).

Although catalase is highly stable in milk (92), the effects of hydrogen peroxide concentration (9, 92), the time of incubation (3), difficulties in stabilizing the concentration of hydrogen peroxide in milk due to catalytic agents such as light (25, 130), heavy metal ions, and lactoperoxidase (75) all have had a marked effect upon the behavior of this enzyme.

CHAPTER III

EXPERIMENTAL PROCEDURE

The methods and procedures outlined in this chapter were directed toward modifying Loane's (74) residual hydrogen peroxide determination so that the resulting test could be used to predict the shelf life of pasteurized milk by testing the raw milk source. The experimental work was divided into two portions, 1) to determine the types of tests to be used in the experiment, and the conditions under which each should be performed, and 2) to collect data for use in demonstrating the relationship of raw milk quality (as measured by the proposed test) to the quality of the resulting pasteurized milk (as measured by conventional methods).

Initially, raw milk samples were collected aseptically in two-liter sterile flasks from the raw milk storage tanks at Texas Tech University and/or from a local dairy firm. All samples were analyzed using the following methods:

1. Titratable acidity
2. Acid degree value
3. Modified residual hydrogen peroxide determination
4. Organoleptic analysis
5. Standard plate count
6. CVT count
7. Proteolytic count
8. Non-protein nitrogen determination
9. Mastitis test (The California Mastitis test was performed on each fresh raw milk sample to check for abnormal milk. Any sample which gave an indication of being mastitic was discarded).

Milk samples were handled by two procedures. In one instance, fresh

milk obtained from the dairy plant was analyzed by the above criteria, and then one-half of the sample was stored at 15 C for 18 hours. The other half of the sample was contaminated with a milkstone source (to be described later) and then incubated at 15 C for 18 hours (in order to obtain samples of milk with high counts). In the other instance, fresh milk after initial analysis was either stored at 4.4 C for 18 hours, or was contaminated with the milkstone source, and then stored at 4.4 C for 18 hours. In both instances, after the 18 hours incubation, the eight mentioned tests were again performed, and the samples were pasteurized and immediately cooled in a crushed ice-saline bath to 4.4 C. After the above mentioned eight determinations were again performed on all milk samples, the pasteurized samples were placed in an incubator at 12.8 C for 6 days (144 hours). Again the same eight tests were performed on each sample, and any sample which did not exhibit an off-flavor at this time was placed back into the 12.8 C incubator and organoleptically scored daily until an off-flavor developed.

An attempt was made to find a correlation or relationship between the shelf life of each raw milk sample (as measured by various chemical, microbial, and organoleptic tests) and results of the modified residual hydrogen peroxide determination.

Standard plate count.

This test gave an indication of the number of bacteria per ml of milk. Bacterial counts of all samples were made according to the procedures recommended by Standard Methods for the Examination of Dairy Products (2).

Modified residual hydrogen peroxide determination.

This test required the use of hydrogen peroxide (H_2O_2), sterile screw cap test tubes (150 x 15 mm), 10-ml fast-delivery sterile pipettes, 40 per cent potassium iodide solution (fresh), and dilution bottles containing 99 ml sterile skim milk. (Care was taken not to store bottles of skim milk beyond 15 days at room temperature since coagulation and serum separation may occur under these conditions (151)).

Ten screw cap sterile test tubes were arranged in a row. Then sterile skim milk was placed in the tubes in amounts ranging from 10 ml in the first tube to 0 ml in the tenth tube (decreasing the amount by one ml in each succeeding tube until there were two ml of skim milk in the ninth tube). Then beginning again with tube number one, test milk was added to each tube in amounts of 0 to 10 ml (increasing the amount by one ml in each succeeding tube with the ninth tube containing 8 ml of test milk). Then, two ml of a hydrogen peroxide solution (made by mixing 8 ml of 3 per cent hydrogen peroxide with 100 ml distilled water) were added to each tube. After shaking, and then incubating 5 hours at 32 C, one ml of 40 per cent potassium iodide (W/V) solution was added to each tube, yellow color development with residual H_2O_2 was observed, and a "catalase activity number" was assigned to the sample to designate at which dilution there was a clearly defined difference in color. For example, if the contents of tube number 7 were yellow and the contents of tube number 8 were white, the "catalase activity number" of that sample was recorded as "7".

Percentage titratable acidity.

This test (2) was used as an indicator of the rate at which lactose was converted to lactic acid as determined by titrating the milk (18 grams) with 0.10 NaOH solution to an end point of pH 8.3 using a Sargent-Welch (Model LSX) pH meter. Duplicate determinations of each sample were performed, and the average of the two determinations was expressed as per cent lactic acid.

Non-protein nitrogen determination.

This test was used to estimate the amount of protein degradation which took place in the stored samples, as determined by micro-Kjeldahl analysis techniques.

> Twenty grams of each milk sample were accurately weighed into a 110 ml graduated cylinder. Thirty ml of 15 per cent trichloroacetic acid were added. The milk-acid mixture was shaken, and distilled water was added to the 100 ml mark. The contents of each graduated cylinder were filtered through number 40 Whatman filter to remove the precipitated protein. Two ml of filtrate were pipetted with a 2 ml volumetric pipette into a 30 ml micro-Kjeldahl flask, and a regular micro-Kjeldahl analysis was then performed.
>
> To perform the micro-Kjeldahl analysis by the "micro-method," the reagents included a) concentrated, nitrogen-free sulfuric acid; b) Digestion mixture -- 3.2 parts copper sulfate and 96.8 parts potassium sulfate; c) Fifty per cent solution of sodium hydroxide; d) Boric acid solution -- Forty grams of boric acid added to one liter of 80 C distilled water; e) Brom cresol green solution (0.10 gram of brom cresol green added to 100 ml distilled water); 0.10 percent solution of methyl red (0.10 gram of methyl red added to 100 ml of 95 per cent ethyl alcohol). (2.5 ml of brom cresol green solution and 1.5 ml of methyl red solution were added to 1 liter of the cooled boric acid solution); f) An exact normality of titer (HCl) was determined which was approximately 0.02 Normal.
>
> To perform the test, a steam distillation apparatus with a water-cooled condenser and a rheostat was used. a) 0.18 gram of digestion mixture was added to the 2 ml filtrate already present in the 30 ml digestion flask; b) Three ml of H_2SO_4 were added to the flask, (for each 10 mg of dry organic matter over 15 grams, an additional 0.1 ml of H_2SO_4 was added) by pouring the acid down the side of the flask to wash down any sample sticking to the neck of the flask; c) The flask was heated to effect proper

digestion as indicated by the development of a clear, green color; d) and after apparent completion of the digestion procedure, heating was continued for an additional 15 minutes.

After digestion, a) The flasks were removed from the digestion racks and cooled to room temperature; b) A minimum of distilled water was added to the flask to dissolve any solids present. (Sometimes, as the sample was cooled some of the salts precipitated out, and if water was not added at this point the sample tended to boil excessively when added to the distillation apparatus); c) After a 50 ml Erlenmeyer flask containing 5 ml of the boric acid indicator solution was placed under the condenser outlet of the distillation apparatus (One should be certain that the tip of the condenser is submerged in the solution), the top petcock of the distillation apparatus was opened, and the digestion mixture was poured in (The flask should be rinsed 4 or 5 times with 1-2 ml portions of the distilled water and added to the distillation apparatus); d) The top of the apparatus was closed and 8-10 ml of concentrated NaOH was poured into the receptacle above the petcock; e) The petcock was opened slightly and the NaOH was allowed to drip in very slowly. (The petcock was closed after the addition of the NaOH, and one or two ml of distilled water were added as a rinse); f) The steam generator was started by setting the rheostat (Staco, Type 1010) on 60 to 65. g) Fifteen ml of the distillate was collected in the flask and removed from beneath the condenser; h) A 150 ml beaker of distilled water was placed under the condenser outlet and the generator was turned off. (As the generator cooled, the water in the beaker was drawn into the apparatus, thus cleaning it. The bottom petcock should be opened in order to allow the water to drain out). i) the distillate was titrated to a gray endpoint (or the first appearance of violet) using 0.02 N HCL. j) A blank determination was made; and k) per cent non-protein nitrogen was calculated by using the formula

$$\frac{(1\text{-}2) \times (3) \times 14 \times 100}{(4)}$$

where 1 = ml of HCl used on sample determination
2 = ml of HCl used on blank determination
3 = normality of HCl
4 = mg of sample

Duplicate sample results were averaged to obtain mg of non-protein nitrogen per 100 ml of milk.

Acid degree value.

This test was used to indicate fat degradation as measured by the fat acidity of samples (39).

A modified Dairy Products Section (DPS) procedure (39) was used for this purpose. The equipment and reagents for this test included a) Standard Babcock centrifuge; b) Water bath. The bath should be gently boiling and should contain enough water to cover the bottle up to the base of the neck; c) Tempering bath. The temperature range should be within 130-140 F and the water level should be at or above the fat column level of the test bottle; d) Two standard 18 gram, 8 per cent Babcock milk test bottles per sample; e) A standard 17.6 ml milk pipette; f) A 50 ml Erlenmeyer flask for each Babcock bottle, plus one flask for the blank; g) A syringe with capacity for at least 5 cc, with a cannula at least 2 inches long and small enough in diameter to fit inside the neck of a milk test bottle; h) A 5.0 ml microburette; i) BDI reagent which consists of thirty grams of Triton-X-100, seventy grams of sodium tetraphosphate, and enough distilled water to make one liter; j) Fifty per cent methanol. Made of equal volumes of pure methanol and distilled water; k) Alcoholic potassium hydroxide (Normality of 0.02); 1) Phenolphthalein indicator solution. m) Petroleum ether; and n) Absolute ethanol.

To perform the test a) Thirty-five ml of milk sample were placed in an 18 gram, 8 per cent Babcock test bottle, using a 17.6 milk pipette; b) Ten ml of BDI reagent were added to the milk sample; and c) The milk and the BDI reagent were mixed thoroughly for about 5 minutes. Then the milk test bottles were placed in a gently boiling water bath for 20 minutes. (The contents were agitated thoroughly every 4 or 5 minutes) and centrifuged for 3-5 minutes.

Enough 50 per cent methanol was added by means of a 17.6 ml milk pipette to bring the top of the fat column to the 7 per cent mark on the neck of the bottle. The contents were again centrifuged for 3-5 minutes, and the milk test bottle was placed in a 130-140 F tempering bath for at least 5 minutes. Using the syringe most of the collected fat was removed from the test bottle and placed into previously weighed 50 ml erlenmeyer flasks, after which the amount of fat in each flask was determined. To the weighed flask, 10 drops of indicator, 10 ml of petroleum ether, and 5 ml of absolute ethanol were added. (A blank determination was also performed). The contents of the sample and blank flasks were titrated to the first definite pink color with the alcoholic KOH solution using a 5.0 ml micro-burette. The results of the titrations were recorded, and acid degree values were calculated, using the formula:

$$\text{Acid degree value} = \frac{(1-2)\ (3)\ (100)}{(4)}$$

where 1 = ml of titer used for the sample
2 = ml of titer used for the blank
3 = normality of the titer
4 = weight in grams of the fat sample

The two results were averaged to obtain a reported acid degree value.

Development of a milkstone source.

Raw milk was added to a three foot long, one and one half inch diameter number 302 stainless steel pipe. The pipe was then heated vigorously until the milk reached a temperature slightly above 180 F. The pipe was then rinsed with a solution of calcium carbonate. This procedure was repeated several times until a desired amount of milkstone had formed.

In order to contaminate a given milk sample, the sample was aseptically placed in the milkstone-contaminated pipe, shaken and aseptically placed in a sterile container. The purpose of this milkstone contamination was to attempt to increase bacterial numbers in the raw milk with a microflora commonly associated with milk processing plants.

Modified CVT test.

This test gave an estimate of the numbers of gram-negative bacteria per ml of milk which developed as red colonies on CVT agar in 48 hours at 32 C.

Materials:

1. Dilution bottles containing 99 ml of sterile skim milk.
2. Standard plate count agar.
3. 0.1 per cent crystal violet solution (0.1 gram crystal

violet in 100 ml distilled water = 1000 p.p.m.)
4. 0.5 per cent alcoholic TTC (0.5 grams triphenyl tetrazolium chloride in 100 ml 95 per cent ethyl alcohol = 5000 p.p.m.).
5. Screw cap test tubes (20 x 150 ml).

The following procedure was used to prepare the CVT agar.

1. The standard plate count agar was rehydrated as per instructions on container.
2. For 4 p.p.m., 4.0 ml of 0.1 per cent crystal violet solution was added per liter of agar.
3. The agar was dispensed in test tubes (about 10 ml per test tube) and sterilized.
4. Two drops of 0.5 per cent TTC were added aseptically per 10 ml of the sterile, melted agar immediately prior to pouring the plates.
5. Serial dilutions were prepared using sterile skim milk (90 grams skim milk powder per 910 ml of distilled water) instead of sterile water.
6. Swirl plates were made and incubated for 48 hours at 32 C.

Since the dye-binding action of the milk proteins had a neutralizing effect upon the crystal violet, it was important to maintain the correct ratio between the dye and the protein. This was found to be about 1 part dye to 750 parts protein with limits of 1-500 and 1-1000(101). Therefore, when performing dilutions of 1:1000 and 1:100,000, it was necessary to add 0.9 ml of sterile skim milk to the petri dish in addition to the 0.1 ml dilution of skim milk and sample already added. This addition gave the correct ratio and also supplied visible casein so that proteolytic counts could be performed. Red colonies were counted and multiplied by the appropriate dilution factor to obtain an indication of the number of gram-negative bacteria per ml of milk.

Proteolytic counts.

When counting CVT plates, clear zones were noticed around certain bacterial colonies. It was assumed that counting these colonies and multiplying the number by the appropriate dilution factor would represent an estimate of the number of proteolytic organisms in the milk.

Organoleptic analysis.

Samples were judged by this author according to flavor, odor, and appearance. Special care was used to detect off-flavors associated with the stored, pasteurized samples.

Analysis of data.

This research included the analyses of 91 samples (including both the raw and pasteurized forms). The pertinent data accumulated were arranged in both tabular and graphical forms in an attempt to detect any relationship which may have existed among the various test results. Emphasis was placed on searching for relationships between the catalase activity numbers of raw milk and indications of short shelf life in the corresponding pasteurized product. Also photographs of the arrangement and color associated with the modified residual hydrogen peroxide determination (catalase activity numbers) were made for illustrative purposes.

CHAPTER IV

RESULTS AND DISCUSSION

Examples of typical visual test results obtained when performing the modified residual hydrogen peroxide determination (catalase activity test) on various raw milk samples are illustrated in Figures 1, 2, 3, and 4. In order to explain the color differences exhibited in these pictures it should be reiterated that tube number 1 (that tube to the left of each picture) contained 10 ml of sterile skim milk; Tube 10 (that tube to the right of each picture) contained 10 ml of test milk, and those tubes between these two extremes contained (from left to right) from 1 through 8 ml (1 ml incremental increases) of test milk respectively. It may be noted in Figures 1 that all tubes exhibited a yellow color while in Figures 2 and 3 some tubes were white. In the case of Figure 1, apparently the sample had such a low bacterial count that the pure test milk turned a yellow color upon testing, while in Figure 2 the milk apparently had such high bacterial counts that the tubes with high milk dilutions remained white. On the other hand, the sample making up the results in Figure 3 apparently had intermediate counts as evidenced by the fact that a line of demarcation could be noted in the dilutions.

A problem which was sometimes encountered in the study namely, that at times the color intensities were neither clear-cut (as in Figure 1, 2, and 3) nor progressively lighter across the tube array

was illustrated in Figure 4. In other words, there would be (from left to right) light color intensities, followed by darker colors, and then another group of tubes with lighter color intensities. No explanations can be offered for these variances. At any rate there were tests in this study which did not conform exactly to the expected reaction pattern, which simply points out the difficulty sometimes encountered in correlating catalase activity determination results with shelf life of pasteurized milk.

In preliminary research, several conditions were tested to determine the best temperature and time parameters to utilize for the test. It was found that a milk incubation temperature and time of 32 C for 5 hours gave the best results. This finding substantiates the work of Loane (74) who, although she found that the temperature range for greatest catalase activity was between 10 and 20 C, concluded that, for milk, a testing temperature of 32 C most nearly correlated with bacterial counts.

In an attempt to correlate results of this test with the shelf life of pasteurized milk, various microbial, chemical, and organoleptic evaluations of the pasteurized product were made. The data shown in Tables 1 and 2 are the results of these tests. The pasteurized milk samples in Table 1 were processed from (i) grade A raw milk from a local dairy (column 1), (ii) portions of the same milk which had been held at 15 C for 18 hours prior to pasteurization (column 2). This milk was then pasteurized (column 3) and stored for 6 days at 12.8 C (column 4), and (iii) another portion of that same milk after it had been contaminated with a milkstone source prior to storage at 15 C

Figure 1. CATALASE ACTIVITY NUMBER OF 10. THIS INDICATED LOW CATALASE ACTIVITY.

Figure 2. CATALASE ACTIVITY NUMBER OF 1. THIS INDICATED VERY HIGH CATALASE ACTIVITY.


JUL 72

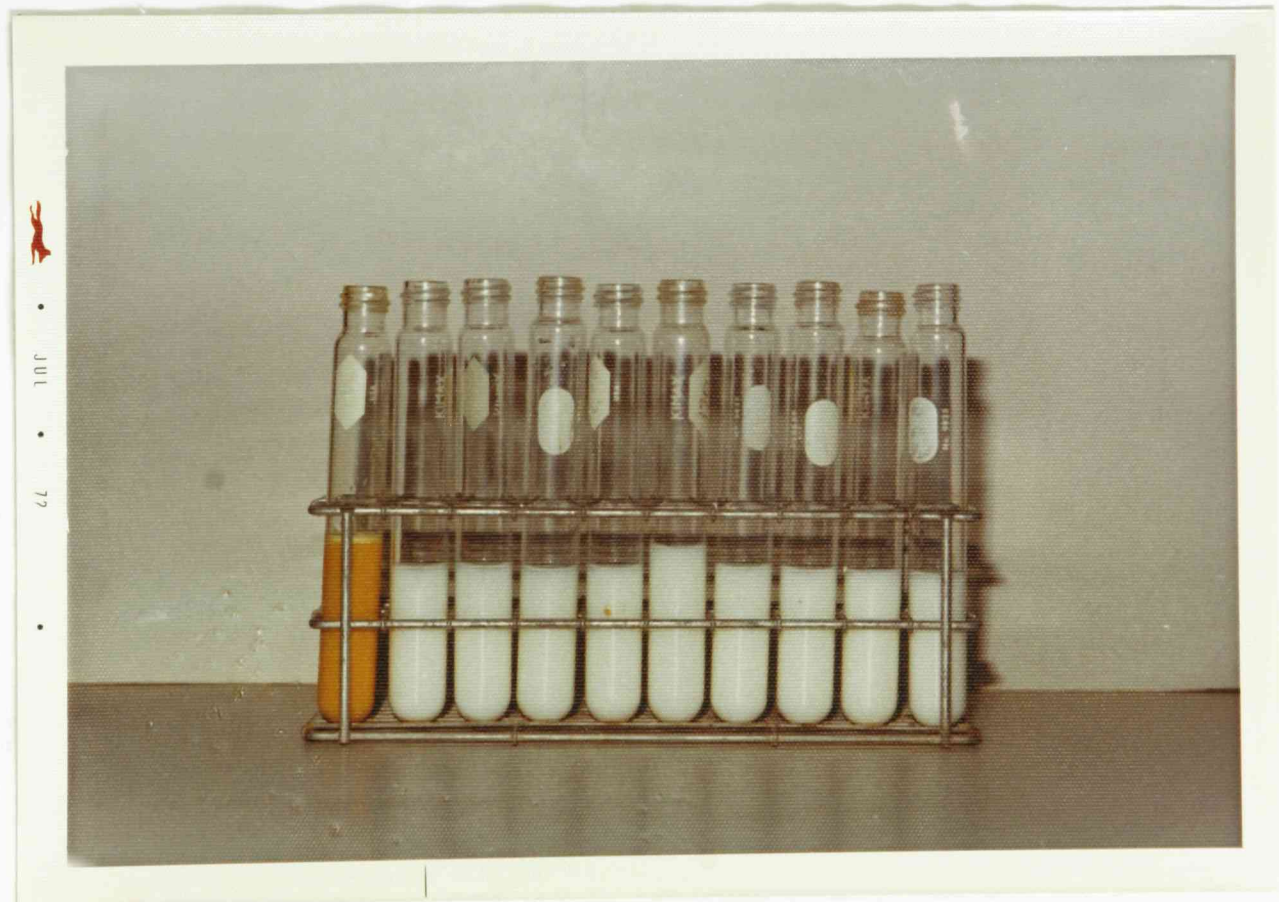
JUL 72

Figure 3. CATALASE ACTIVITY NUMBER OF 5. THIS PHOTOGRAPH ILLUSTRATES AN EXCELLENT DIFFERENTIATION BETWEEN THE CONTENTS OF TEST TUBES 5 AND 6.

Figure 4. CATALASE ACTIVITY NUMBER OF 7. THIS PHOTOGRAPH ILLUSTRATES A POOR COLOR DIFFERENTIATION BETWEEN THE CONTENTS OF TEST TUBES 5 AND 8.


JUL 72


JUL 72

TABLE I

SAMPLE ONE: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.17	0.16	0.17	0.17	0.17	0.17	0.17
CATALASE ACTIVITY NUMBERS	10	4	10	5	4	10	5
ACID DEGREE VALUE	1.10	1.20	1.06	1.30	1.11	0.81	1.34
STANDARD PLATE COUNT (Bacteria/ml)	47,000	980,000	Less than 100	1,125,000	1,190,000	Less than 100	1,113,000
CVT*COUNT (Bacteria/ml)	38,000	3,400,000	Less than 10	3,900,000	3,700,000	Less than 10	4,100,000
PROTEOLYTIC COUNT (Bacteria/ml)	4,100	19,300	Less than 10	105,000	21,400	Less than 10	193,000
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	23.10	24.85	24.85	28.70	27.30	27.75	29.90
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Bitter	No Criticism	No Criticism	Bitter

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE TWO: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATION		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.17	0.17	0.17	0.17	0.18	0.17	0.17
CATALASE ACTIVITY NUMBERS	10	7	10	4	1	10	4
ACID DEGREE VALUE	1.19	1.21	0.83	1.62	1.23	0.89	1.45
STANDARD PLATE COUNT (Bacteria/ml)	128,000	4,600,000	Less than 100	2,010,000	6,900,000	Less than 100	2,500,000
CVT*COUNT (Bacteria/ml)	101,000	7,400,000	111	3,300,000	9,750,000	410	4,600,000
PROTEOLYTIC COUNT (Bacteria/ml)	31,500	29,500	Less than 10	99,000	87,500	Less than 10	253,000
NITROGEN ANALYSIS (mg/100 g. milk)	28.05	36.50	31.20	38.95	37.35	37.15	39.90
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Stale after 5 days	No Criticism	Slightly Cooked	Bitter and Fruity

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE THREE: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.18	0.18	0.18	0.18	0.17	0.17	0.18
CATALASE ACTIVITY NUMBERS	10	4	10	4	3	10	1
ACID DEGREE VALUE	1.00	1.20	1.10	1.75	1.15	1.12	1.79
STANDARD PLATE COUNT (Bacteria/ml)	110,000	27,900,000	154	33,000,000	24,700,000	137	29,100,000
CVT*COUNT (Bacteria/ml)	67,500	28,900,000	Less than 10	37,000,000	36,300,000	Less than 10	34,000,000
PROTEOLYTIC COUNT (Bacteria/ml)	7,000	77,500	Less than 10	222,000	115,000	Less than 10	236,000
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	34.40	37.35	37.30	40.00	39.35	37.65	42.35
FLAVOR SCORE	No Criticism	No Criticism	Slightly Cooked	Bitter after 4 days	No Criticism	No Criticism	Bitter after 4 days

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE FOUR: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.15	0.14	0.14	0.17	0.16	0.15	0.19
CATALASE ACTIVITY NUMBERS	10	5	10	10	5	10	10
ACID DEGREE VALUE	1.01	1.18	0.86	1.24	1.10	0.83	1.14
STANDARD PLATE COUNT (Bacteria/ml)	97,000	1,600,000	Less than 100	3,400,000	8,150,000	Less than 100	3,990,000
CVT*COUNT (Bacteria/ml)	33,250	1,600,000	Less than 10	Less than 10	1,330,000	Less than 10	Less than 10
PROTEOLYTIC COUNT (Bacteria/ml)	1,500	70,000	Less than 10	Less than 10	117,000	Less than 10	Less than 10
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	33.45	36.80	36.55	37.95	37.00	36.30	37.55
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Stale and Bitter	No Criticism	No Criticism	Stale and Bitter

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE FIVE: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.17	0.16	0.15	0.15	0.17	0.16	0.16
CATALASE ACTIVITY NUMBERS	10	7	10	6	1	10	5
ACID DEGREE VALUE	1.01	1.12	0.99	1.20	1.17	0.98	1.40
STANDARD PLATE COUNT (Bacteria/ml)	83,000	3,200,000	Less than 100	1,940,000	7,200,000	127	2,930,000
CVT*COUNT (Bacteria/ml)	49,000	3,400,000	Less than 10	3,950,000	8,800,000	Less than 10	4,100,000
PROTEOLYTIC COUNT (Bacteria/ml)	7,000	150,000	Less than 10	94,000	242,000	Less than 10	139,000
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	22.50	26.00	23.00	26.65	26.60	25.90	30.80
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Stale	Fruity	Fruity	Fruity

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE SIX: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Condition	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.16	0.17	0.15	0.18	0.16	0.15	0.18
CATALASE ACTIVITY NUMBERS	10	4	10	4	4	10	4
ACID DEGREE VALUE	0.57	0.57	0.45	0.65	0.58	0.48	0.69
STANDARD PLATE COUNT (Bacteria/ml)	325,000	11,800,000	208	2,460,000	11,200,000	313	1,970,000
CVT*COUNT (Bacteria/ml)	319,000	16,000,000	Less than 10	Less than 10	18,000,000	1	Less than 10
PROTEOLYTIC COUNT (Bacteria/ml)	33,000	550,000	Less than 10	Less than 10	750,000	Less than 10	Less than 10
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	30.80	31.05	30.55	32.80	34.70	33.70	34.75
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Sour	No Criticism	No Criticism	Sour

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE SEVEN: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.14	0.14	0.14	0.15	0.14	0.14	0.15
CATALASE ACTIVITY NUMBERS	10	6	10	7	4	10	8
ACID DEGREE VALUE	0.96	0.99	0.98	1.60	0.97	0.96	1.33
STANDARD PLATE COUNT (Bacteria/ml)	8,150	1,930,000	Less than 100	6,300,000	2,550,000	Less than 100	7,500,000
CVT*COUNT (Bacteria/ml)	5,400	975,000	Less than 10	9,600,000	1,430,000	Less than 10	11,000,000
PROTEOLYTIC COUNT (Bacteria/ml)	2,600	50,000	Less than 10	11,000	36,000	Less than 10	33,000
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	30.15	35.50	37.00	38.50	37.25	39.30	46.90
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Bitter after 5 days	No Criticism	No Criticism	Fruity after 5 days

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE EIGHT: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.15	0.15	0.15	0.17	0.15	0.15	0.17
CATALASE ACTIVITY NUMBERS	10	5	10	9	4	10	9
ACID DEGREE VALUE	0.63	0.84	0.99	0.89	0.83	0.83	1.13
STANDARD PLATE COUNT (Bacteria/ml)	37,000	512,000	Less than 100	2,310,000	560,000	Less than 100	3,250,000
CVT*COUNT (Bacteria/ml)	7,000	1,440,000	Less than 10	71,000	1,930,000	Less than 10	140,000
PROTEOLYTIC COUNT (Bacteria/ml)	150	29,700	Less than 10	Less than 10	70,000	Less than 10	Less than 10
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	29.65	33.00	33.40	41.30	33.90	34.25	43.00
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Fruity after 5 days	No Criticism	No Criticism	Fruity after 5 days

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE NINE: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
	0-HOURS	RAW	PASTEURIZED	PASTEURIZED	RAW	PASTEURIZED	PASTEURIZED
		18-Hours	0-Hours	144-Hours	18-Hours	0-Hours	144-Hours
Tests		15 C		12.8 C	15 C		12.8 C
TITRATABLE ACIDITY (%)	0.16	0.16	0.15	0.16	0.16	0.5	0.17
CATALASE ACTIVITY NUMBERS	10	6	10	8	6	10	8
ACID DEGREE VALUE	1.11	1.23	1.22	1.51	1.27	1.25	1.68
STANDARD PLATE COUNT (Bacteria/ml)	340,000	17,000,000	Less than 100	7,400,000	17,300,000	Less than 100	6,600,000
CVT*COUNT (Bacteria/ml)	116,000	26,000,000	Less than 10	12,600,000	25,400,000	Less than 10	14,700,000
PROTEOLYTIC COUNT (Bacteria/ml)	35,000	400,000	Less than 10	845,000	269,000	Less than 10	700,000
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	23.10	27.30	25.20	28.15	27.80	26.45	29.90
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Bitter after 4 days	No Criticism	No Criticism	Very Bitter after 4 days

*Crystal violet-tetrazolium chloride

TABLE I -- Continued

SAMPLE TEN: EXPERIMENTAL DATA ASSOCIATED WITH SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.15	0.17	0.16	0.17	0.17	0.16	0.18
CATALASE ACTIVITY NUMBERS	10	6	10	7	6	10	6
ACID DEGREE VALUE	0.97	1.08	0.99	1.22	1.12	0.97	1.40
STANDARD PLATE COUNT (Bacteria/ml)	7,750	1,250,000	Less than 10	255,000	1,100,000	Less than 100	435,000
CVT*COUNT (Bacteria/ml)	6,100	985,000	Less than 10	Less than 10	735,000	Less than 10	Less than 10
PROTEOLYTIC COUNT (Bacteria/ml)	800	100,000	Less than 10	Less than 10	95,000	Less than 10	Less than 10
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	35.00	45.50	46.20	48.30	45.50	44.10	49.70
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	Stale and Unclean	No Criticism	No Criticism	Stale and Unclean

*Crystal violet-tetrazolium chloride

TABLE II

SAMPLE ELEVEN: EXPERIMENTAL DATA FOR SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.16	0.16	0.15	0.16	0.16	0.16	0.16
CATALASE ACTIVITY NUMBERS	10	10	10	10	10	10	10
ACID DEGREE VALUE	0.78	0.94	0.90	0.95	0.94	0.91	0.99
STANDARD PLATE COUNT (Bacteria/ml)	36,500	33,500	Less than 100	9,000	39,000	Less than 100	9,600
CVT*COUNT (Bacteria/ml)	7,900	8,000	Less than 10	Less than 10	12,000	Less than 10	Less than 10
PROTEOLYTIC COUNT (Bacteria/ml)	300	250	Less than 10	Less than 10	1,230	Less than 10	Less than 10
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	31.05	29.40	33.45	33.95	30.80	32.00	36.85
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	No Criticism	No Criticism	No Criticism	No Criticism
OFF-FLAVOR OCCURRENCE	10 days = oxidized				10 days = Fruity		

*Crystal violet-tetrazolium chloride

TABLE II -- Continued

SAMPLE TWELVE: EXPERIMENTAL DATA FOR SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.14	0.14	0.14	0.15	0.14	0.14	0.15
CATALASE ACTIVITY NUMBERS	10+	10+	10+	10+	10+	10+	10+
ACID DEGREE VALUE	0.96	1.15	1.00	1.01	0.98	0.96	1.21
STANDARD PLATE COUNT (Bacteria/ml)	8,500	10,100	Less than 100	55,700	17,400	Less than 100	51,100
CVT*COUNT (Bacteria/ml)	5,800	5,500	Less than 10	62,000	7,800	Less than 10	72,000
PROTEOLYTIC COUNT (Bacteria/ml)	2,900	1,600	Less than 10	1,000	3,700	Less than 10	2,000
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	31.50	34.80	35.00	38.25	36.00	36.05	37.90
FLAVOR SCORE	No Criticism	No Criticism	Cooked	No Criticism	No Criticism	Cooked	No Criticism
OFF-FLAVOR OCCURRENCE	9 days = Oxidized				9 days = Oxidized		

*Crystal violet-tetrazolium chloride

TABLE II -- Continued

SAMPLE THIRTEEN: EXPERIMENTAL DATA FOR SHELF LIFE PREDICTION TEST

COLUMN:	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Conditions	RAW	NON-CONTAMINATED			MILKSTONE CONTAMINATED		
Tests	0-HOURS	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C	RAW 18-Hours 15 C	PASTEURIZED 0-Hours	PASTEURIZED 144-Hours 12.8 C
TITRATABLE ACIDITY (%)	0.14	0.13	0.14	0.14	0.13	0.14	0.14
CATALASE ACTIVITY NUMBERS	10	10	10	10	10	10	10
ACID DEGREE VALUE	0.68	0.63	0.62	0.71	0.65	0.59	0.70
STANDARD PLATE COUNT (Bacteria/ml)	37,000	34,000	Less than 100	13,000	39,000	Less than 100	12,000
CVT*COUNT (Bacteria/ml)	12,000	17,000	Less than 10	Less than 10	16,000	Less than 10	Less than 10
PROTEOLYTIC COUNT (Bacteria/ml)	1,000	1,000	Less than 10	Less than 10	1,000	2,000	Less than 10
NON-PROTEIN NITROGEN ANALYSIS (mg/100 g. milk)	26.00	29.20	30.00	32.00	26.05	26.10	27.85
FLAVOR SCORE	No Criticism	No Criticism	No Criticism	No Criticism	No Criticism	No Criticism	No Criticism
OFF-FLAVOR OCCURRENCE	8 days = stale				8 days = stale		

*Crystal violet-tetrazolium chloride

for 18 hours (column 5). This milk was pasteurized (column 6) and stored for 6 days at 12.8 C (column 7). Table 2 was identical to Table 1 except that the 18 hour incubation period (columns 2 and 5) was at 4.4 C instead of 15 C.

As may be noted from these two tables, although there was no appreciable difference between the titratable acidities of the raw and freshly pasteurized milks, the acidity of some of the stored pasteurized samples was higher. Also, the acid degree values and non-protein nitrogen contents of the stored product were higher than those of the milk in its raw state. However, no relationship could be found between the catalase activity numbers (of raw, incubated, freshly pasteurized, or stored-pasteurized samples) and any of these chemical test results.

For example, no linear relationship between catalase activity numbers of the raw milk after an 18 hour storage period at 15 C and either titratable acidity, acid degree value or non-protein nitrogen after the sample had been pasteurized and stored for 6 days at 12.8 C was evident in Figures 5, 6, and 7 respectively. Furthermore, the standard plate counts, proteolytic bacterial counts, and CVT counts were higher in many stored samples than in the other milk samples. Again no linear relationships could be found between any of the sets of catalase activity numbers and these counts (Figures 8, 9, and 10).

Catalase activity numbers of raw milk which had been held for 18 hours prior to pasteurization at either 15 C or 4.4 C (columns 2 and 5 of Tables I and II respectively) were compared to flavor of the resulting pasteurized products after storage for six days at 12.8 C. The following

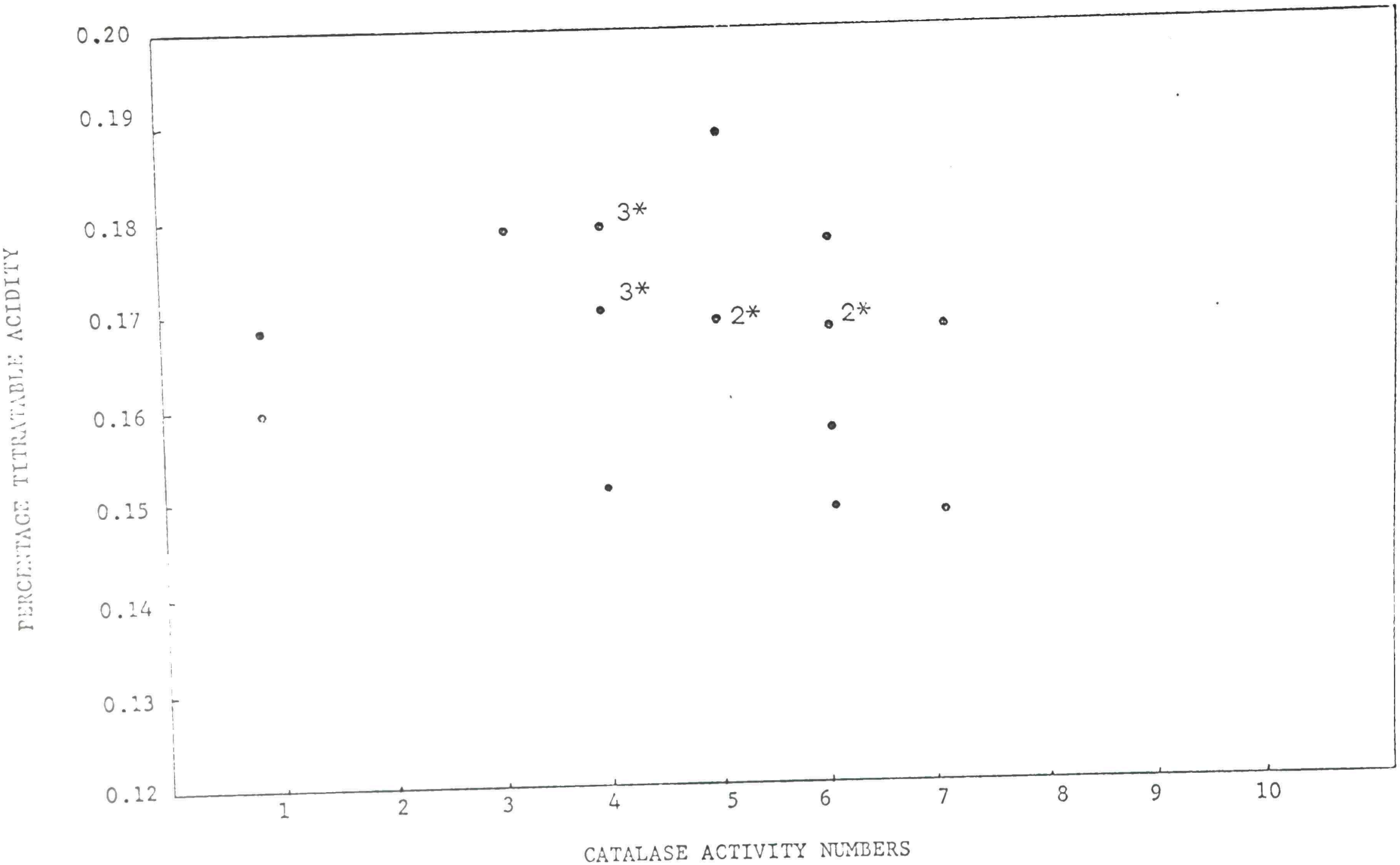

Fig. 5. A Comparison of Percentage Titratable Acidity of the Pasteurized Samples after a 6 day Incubation Period at 12.8 C vs Catalase Activity Numbers of the Raw Samples at the end of an 18 hour Incubation Period at 15 C.

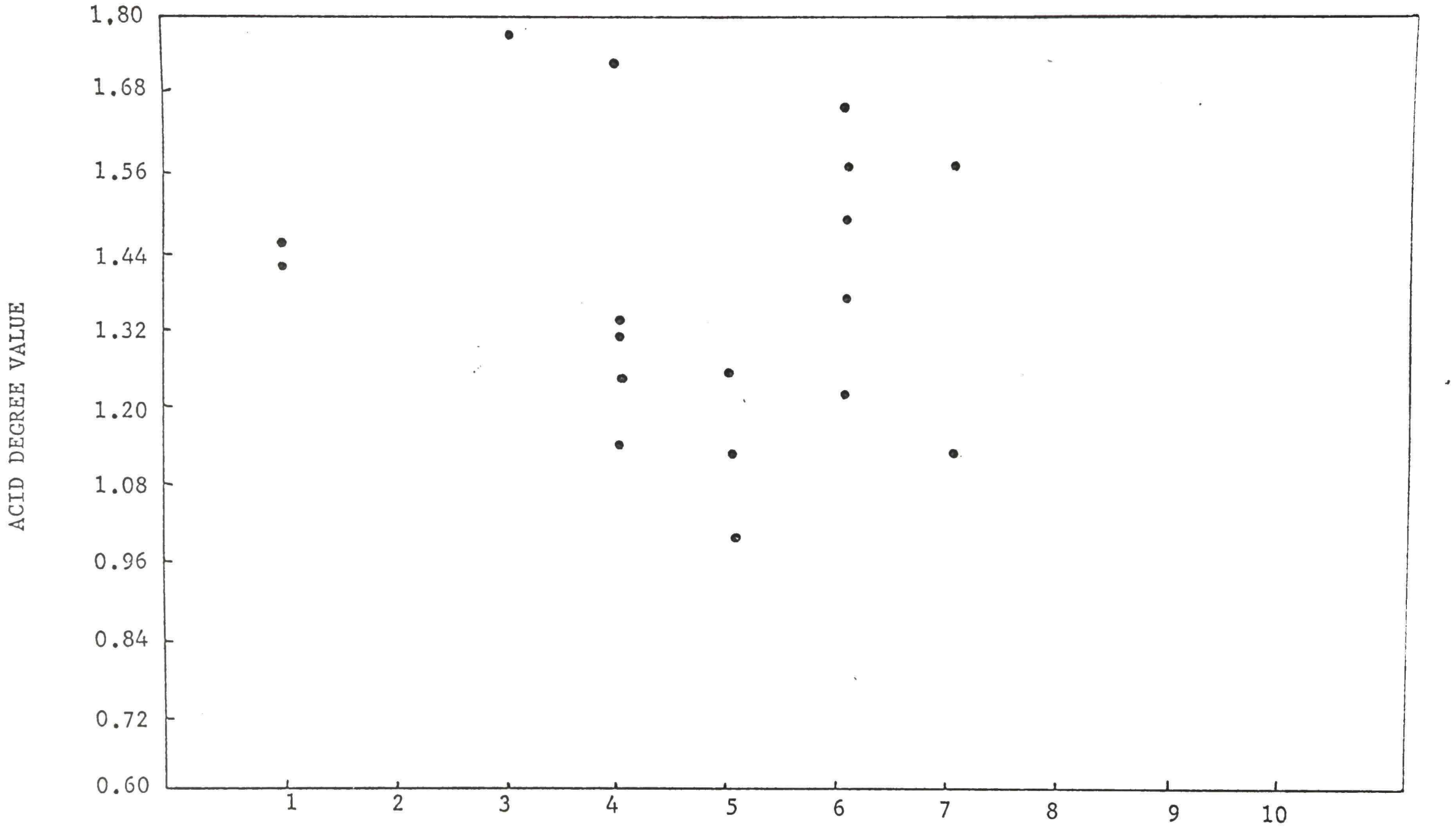

Fig. 6. A Comparison of Acid Degree Values of the Pasteurized Samples after a 6 day Incubation Period at 12.8 C vs Catalase Activity Numbers of the Raw Samples at the end of an 18 hour Incubation Period at 15 C.

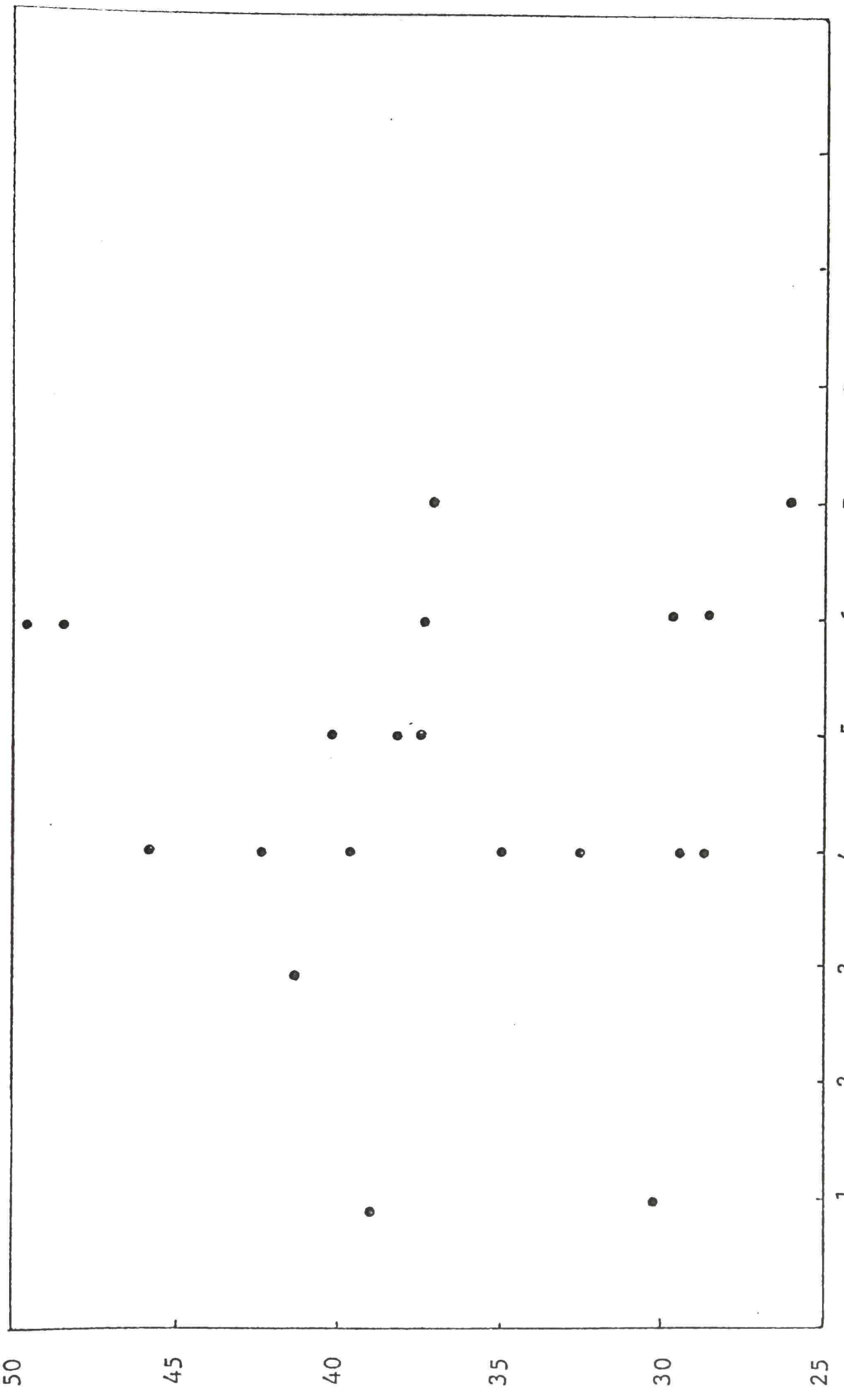

Fig. 7. A Comparison of Non-Protein Nitrogran Values (mg/100 ml milk) of the Pasteurized Samples after a 6 day Incubation Period at 12.8 C vs Catalase Activity Numbers of the Raw Samples at the end of an 18 hour Incubation Period at 15 C.

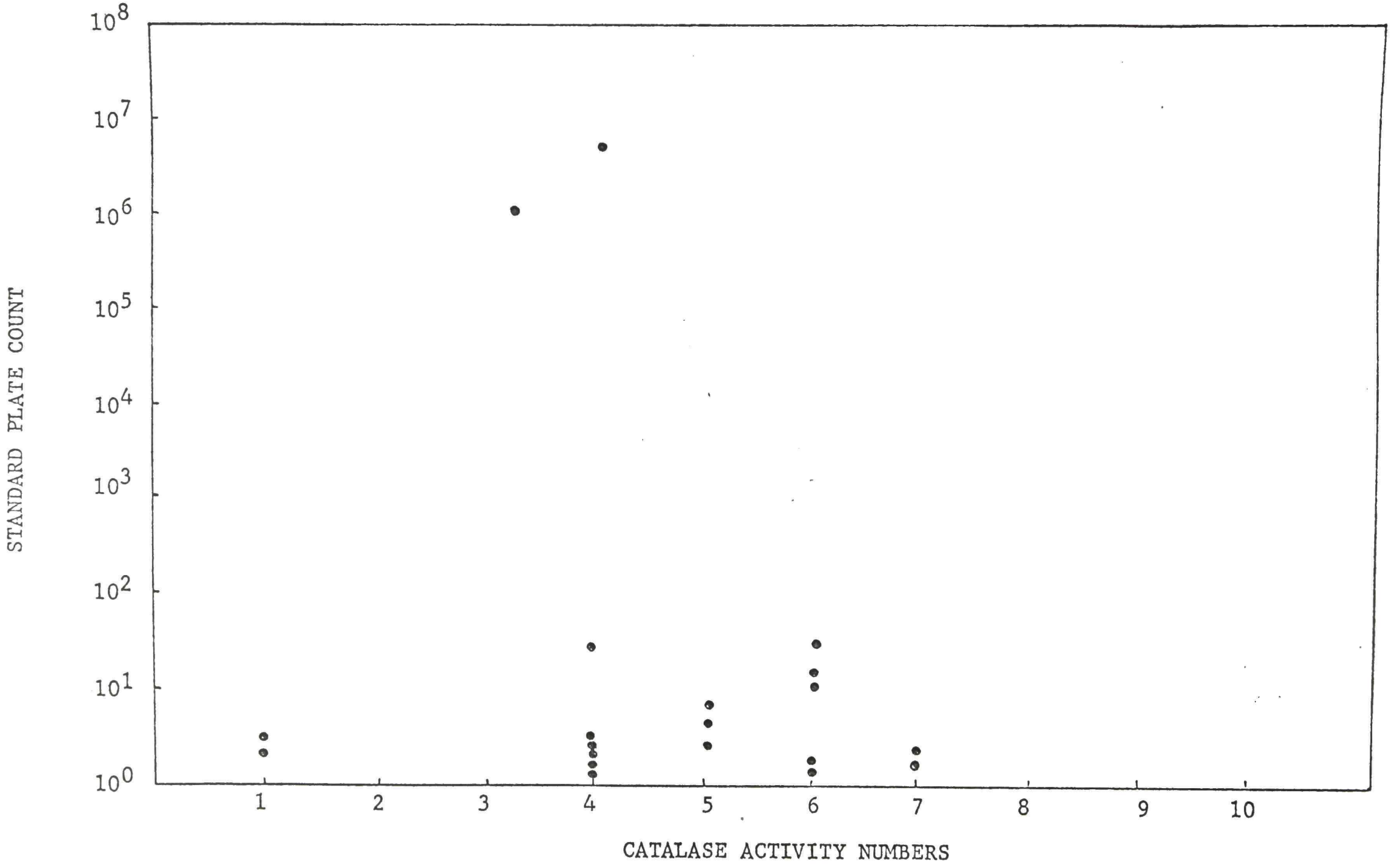

Fig. 8. A Comparison of the Standard Plate Count (millions/ml) of the Pasteurized Samples after a 6 Day Incubation Period at 12.8 C vs. Catalase Activity Numbers of the Raw Samples at the end of an 18 hour Incubation Period at 15 C.

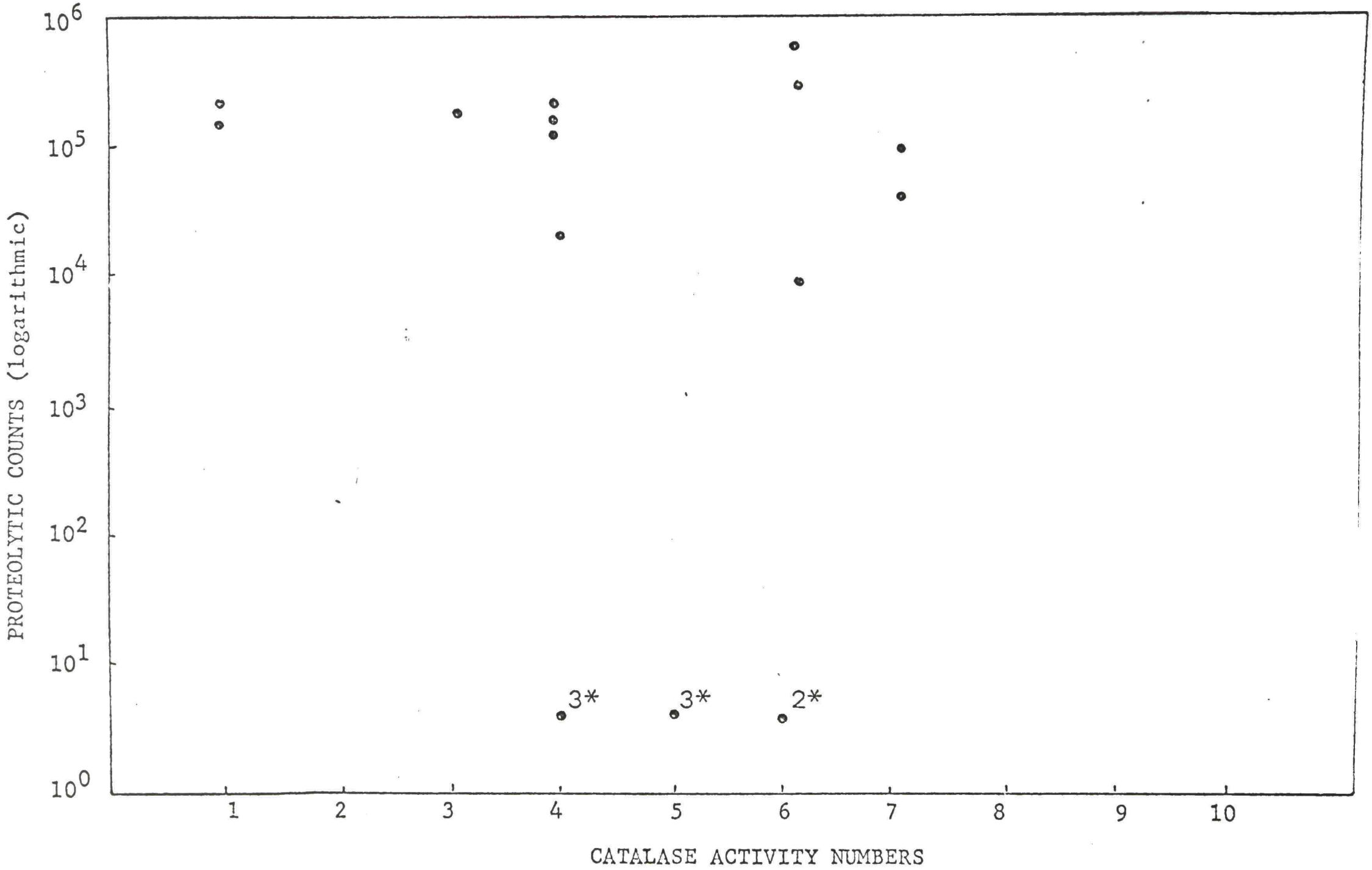

Fig. 9. A Comparison of the Proteolytic Counts (logarithmic) of the Pasteurized Samples after a 6 Day Incubation Period at 12.8 C vs. Catalase Activity Numbers of the Raw Samples at the end of an 18 hour Incubation Period at 15 C.

*Indicates number of samples that contained less than 10 bacteria per ml.

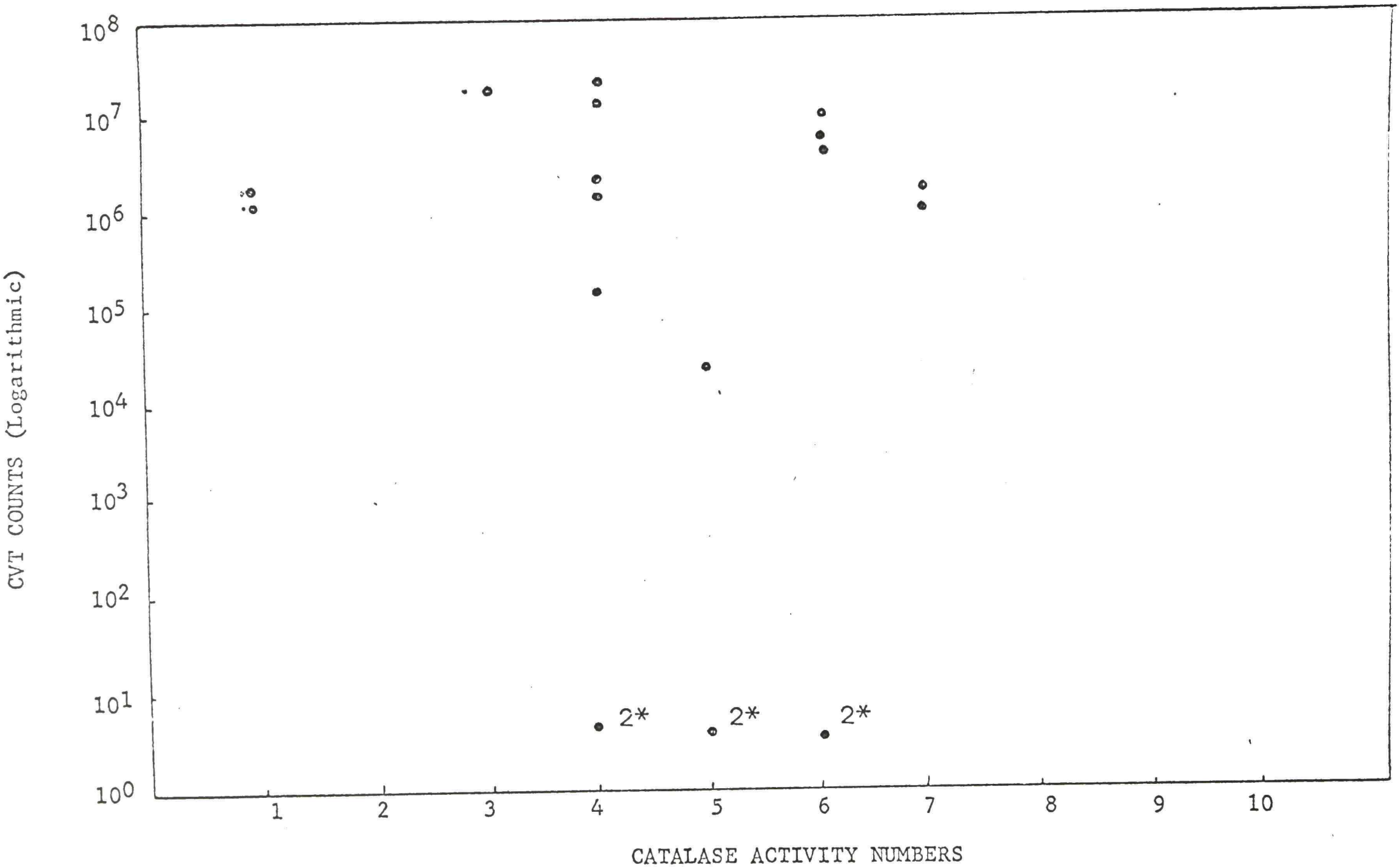

Fig. 10. A Comparison of the CVT Count of the Pasteurized Samples after a 6 day Incubation Period at 12.8 C vs. Catalase Activity Numbers of the Raw Samples at the end of an 18 hour Incubation Period at 15 C.

*Indicates number of samples at these coordinates.

are the results of that comparison:

Catalase Activity Numbers	Flavors after 6 days storage at 12.8 C
1	1 Fruity
3	1 Bitter
4	4 Bitter, 3 Fruity, and 2 Sour
5	2 Stale and 1 Fruity
6	3 Bitter, 2 Stale, and 2 Unclean
7	2 Stale
10	6 No Criticism

From these data, it would appear that pasteurized samples associated with catalase activity numbers of 10 on the raw milk can be expected to have no appreciable flavor criticisms for at least 6 days storage at 12.8 C and that catalase activity numbers of 7 or less indicate that off-flavors may develop within 6 days. It must be kept in mind that the samples associated with catalase activity numbers of 10 were all from raw milk which had been incubated 18 hours at 4.4 C rather than at 15 C; possibly this only points out an additional reason for being careful to keep raw milk cold during storage.

In connection with this apparent relationship between off-flavor development and catalase activity numbers, an additional comparison was made between catalase activity numbers of incubated raw samples and shelf life of the resulting pasteurized product (the number of days before a noticeable off-flavor developed). From figure 11, those samples from raw milks with catalase activity numbers of 10 had a shelf life

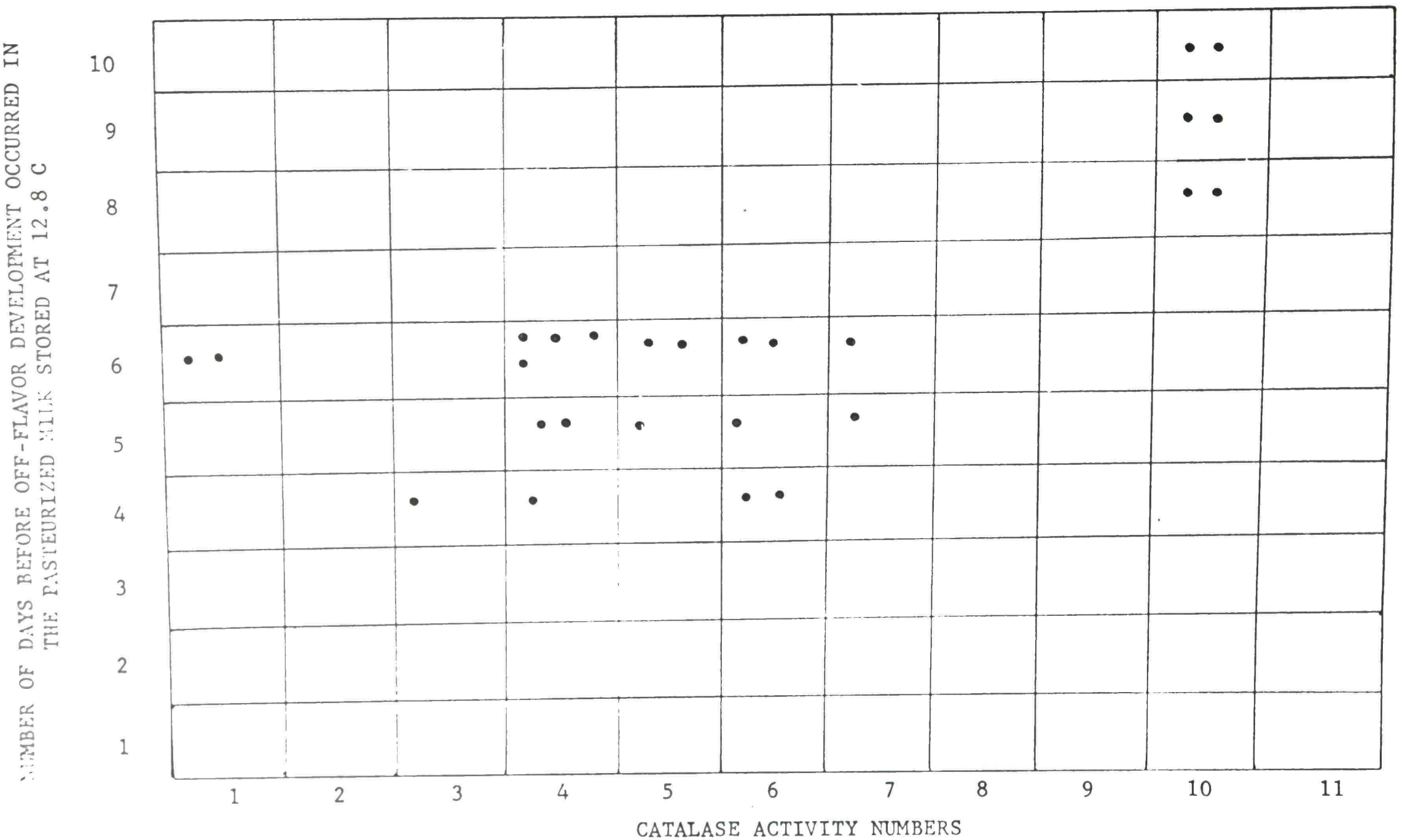

Fig. 11. A Comparison of the Number of Days Required before Off-Flavor Development Occurred in the Stored Pasteurized Samples (12.8 C) vs. Catalase Activity Numbers after the Raw Milk had been Incubated for 18 hours at 15 C.

of 8 to 10 days while catalase activity numbers of less than 10 resulted in a shelf life of 6 or less days.

Therefore, the use of the modified residual hydrogen peroxide determination (catalase activity test) on raw milk sources has at least limited possibilities as a test for predicting the shelf life of dairy products made from these sources. This conclusion should not be construed as meaning there is a direct relationship between poor, medium, or good shelf life and low, medium, or high catalase activity numbers. Rather, the test may be of value in distinguishing between low quality and high quality raw milks with respect to the potential shelf life of those milks after processing.

CHAPTER V

SUMMARY

The dairy industry is in need of a test that is reliable for predicting the shelf life of pasteurized milks. This research was coordinated in an attempt to develop such a test.

A review of past research was made in an effort to gain the expertise necessary for developing a procedure which could be performed upon the milk in its raw state to predict the shelf life of the milk after pasteurization and refrigerated storage. A search of the literature revealed that the enzyme catalase is produced by psychrophilic bacteria commonly associated with off-flavor development in pasteurized, refrigerated milk. Loane (74) discovered that there was a positive correlation between a test which measured catalase activity in raw milk and arbitrary bacterial standards for a satisfactory milk. A positive correlation also existed between the test and organoleptic grading of the milk.

Accordingly, the objective of this study was to modify a catalase activity test so that when it was performed on raw milk, the results would predict the shelf life of the pasteurized product. In order to analyze the effectiveness of such a test, conventional methods were utilized to find any linear or curvilinear relationship which might exist between the modified residual hydrogen peroxide determination ("catalase activity numbers") and the shelf life of the pasteurized milk.

"Catalase activity numbers" of raw milk did not seem to correlate with changes in composition of pasteurized milk in storage with respect to titratable acidity, non-protein nitrogen content, and free-fatty acid degree. Neither was there an apparent direct linear relationship between "catalase activity numbers" and degrees of shelf life quality of pasteurized milk in terms of days in storage before significant off-flavor development. However, the test demonstrated a limited capacity to distinguish between low and high quality raw milk with respect to the potential shelf life of those milks after processing.

Therefore, it was concluded that the test has merits as a possible tool for evaluating certain quality characteristics of raw milk supplies, and that further studies on the subject could result in achieving the long sought goal of finding a raw-milk test, the results of which would correlate with shelf life of market milk.

LIST OF REFERENCES

1. Alexander, Hazel, and C. Higgenbottom. 1953. Bacteriological studies on pasteurized milk. J. Dairy Res. 20:156.

2. American Public Health Association. 1960. Standard Methods for the Examination of Dairy Products. American Public Health Association, Inc., New York.

3. Amin, V. M., and N. F. Olson. 1967. Effect of temperature on stability of hydrogen peroxide in milk. J. Dairy Sci. 50:1336.

4. Andrey, J. J., and W. C. Frazier. 1959. Psychrophiles in milk held two days in farm bulk cooling tanks. J. Dairy Sci. 42:1781.

5. Ashton, T. R. 1950. Some bacteriological aspects of the deterioration of pasteurized milk. J. Dairy Sci. 17:261.

6. Atherton, H. V., F. J. Doan, and G. W. Watrous. 1953. Observations on bacterial populations and characteristics of bottled milk under refrigerated holding. J. Dairy Sci. 36:570.

7. Atherton, H. V., and A. Bradfield. 1957. An evaluation of milk quality as influenced by daily vs. every-other-day pick-up of milk cooled in farm bulk tanks. J. Milk Food Technol. 20:223.

8. Babel, F. J., E. B. Collins, J. C. Olson, I. I. Peters, G. H. Watrous, and M. L. Speck. 1955. The standard plate count of milk as affected by the temperature of incubation. J. Dairy Sci. 38:499.

9. Babel, F. J., and B. W. Hammer. 1957. Dairy Bacteriology. John Wilkins and Sons, Inc., New York. 614 p.

10. Babel, F. J., and R. E. Willets. 1965. Relationship of catalase activity to the leucocyte count of milk. J. Dairy Sci. 48:768.

11. Barnard, S. E. 1968. Acid-degree values of store purchased pasteurized milk. J. Dairy Sci. 51:987.

12. _____. 1968. The importance of shelf-life of milk to consumer acceptance. Dairy Field 154:51.

13. Barnhart, H. M., R. B. Maxcy, and C. E. Georgi. 1970. Effect of humidity on the fate of the microflora of milk on stainless steel surfaces. Food Technol. 24:1385.

14. Barnum, H. J. 1969. The evils of the standard plate count. J. Milk Food Technol. 32:219.

15. Blankengal, G., and E. S. Humbert. 1965. Observations on keeping quality of low bacterial count milk. Can.Dairy Ice Cream J. 44:16.

16. Boyd, J. C., C. K. Smith, and G. M. Trout. 1953. The role of psychrophilic bacteria in the keeping quality of commercially pasteurized and homogenized milk. J. Dairy Sci. 36:571.

17. Breed, R. S., E. G. D. Murry, and N. R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Co., Baltimore. 1094 p.

18. Broitman, S., W. L. Mallman, and G. M. Trout. 1958. A simple test for detecting keeping quality for milk. J. Milk Food Technol. 21:280.

19. Burgwald, L. H., and P. V. Josephson. 1946. Consideration on the keeping quality of pasteurized milk. Am. Milk Review 8:46.

20. _____. 1947. The effect of refrigeration on the keeping quality of pasteurized milk. J. Dairy Sci. 30:383.

21. Cannon, R. Y. 1970. Types and populations of microorganisms in the air of fluid milk plants. J. Milk Food Technol. 33:19.

22. Chaudhari, N. K. 1965. Studies on methods for predicting the shelf life of pasteurized milk products. Unpublished M. S. thesis. Oklahoma State University, Stillwater.

23. Collins, E. B. 1967. Use of preservatives in milk and dairy products. J. Dairy Sci. 50:599.

24. Courtney, J. L. 1963. The effect on bacterial counts of the gradual warming of milk samples. J. Milk Food Technol. 26:389.

25. Curran, H. R., E. R. Evans, and A. Leviton. 1940. The sporicidal action of hydrogen peroxide and the use of crystalline catalase to dissipate residual peroxides. J. Bacteriol. 40:423.

26. Dabbah, R., W. A. Moats, and J. F. Mattich. 1968. Some factors involved in the recovery of psychrophilic bacteria following sub-lethal heat treatment. J. Dairy Sci. 52:927. (abstr.).

27. _____. 1969. Factors affecting resistance to heat and recovery of heat injured bacteria. J. Dairy Sci. 52:608.

28. Dahlberg, A. C. 1945. The keeping quality of pasteurized milk in the New York Metropolitan area during cool weather as determined by bacterial counts, presence of coliform bacteria and flavor. J. Dairy Sci. 28:779.

29. _____. 1946. The relationship of the growth of all bacteria and coliform bacteria in pasteurized milk held at refrigeration temperatures. J. Dairy Sci. 29:651.

30. Day, E. A., and F. J. Doan. 1956. A test for the keeping quality of pasteurized milk. J. Milk Food Technol. 19:53.

31. Edwards, J. D. 1965. An evaluation of the crystal-violet tetrazolium chloride test for predicting shelf-life of selected dairy products. M. S. thesis. Texas Technological College, Lubbock.

32. Egdel, J. W., and E. R. Bird. 1950. Types of organisms present in commercially pasteurized milk. J. Soc. of Dairy Technol. 3:171.

33. Elliker, P. R. 1968. Shelf-life of food products. Am. Dairy Rev. 30:80.

34. Elliker, P. R., E. L. Sing, L. J. Christensen, and W. E. Sandine. 1968. Psychrophilic bacteria and the keeping quality of pasteurized dairy products. J. Milk Food Technol. 31:141.

35. Fabian, F. W. 1946. Significance of the thermoduric and thermophilic bacteria in milk and their control. J. Milk Food Technol. 9:273.

36. Finley, R. D., H. B. Warren, and R. E. Hargrove. 1968. Storage stability of commercial milk. J. Milk Food Technol. 31:382.

37. Ford, H. F., and F. J. Babel. 1959. Milk quality problems associated with present day marketing. J. Milk Food Technol. 22:141.

38. Furia, T. E. 1968. Handbook of Food Additives. The Chemical Rubber Co., Cleveland, Ohio. 700 p.

39. Garrison, W. G. 1969. Relationship of bacterial spore counts in raw milk to shelf life of pasteurized products. M. S. thesis. Texas Tech University, Lubbock.

40. Garvie, E. I. 1955. The growth of E. coli in buffer substrate and distilled water. J. Bacteriol. 69:303.

41. Glazier, L. R. 1963. Quality comparison of high-temperature, short-time and ultra-high temperature pasteurized milk. J. Milk Food Technol. 26:347.

42. Glenn, W. E. 1958. Relationship of bacterial types and numbers to spoilage of pasteurized milk at certain refrigerator temperatures. J. Dairy Sci. 41:346.

43. Grosshopf, J. C., and W. J. Harper. 1960. Role of psychrophilic spore-formers in long life milk. J. Dairy Sci. 52:897 (abstr.).

44. Gunsalus, I. C., B. L. Horecker, and W. A. Wood. 1955. Pathways of carbohydrate metabolism in microorganisms. Bacteriol. Revs. 19:222.

45. Hammer, B. W., and R. H. Hix. 1916. Studies on the numbers of bacteria in milk showing various changes. Iowa Agr. Exp. Sta. Res. Bull 29, June 1916. Ames, Iowa.

46. Hankin, L., and W. F. Dillman. 1968. A rapid test to find potentially psychrophilic organisms in pasteurized dairy products. J. Milk Food Technol. 31:141.

47. Hartley, J. C., G. W. Reinbold, and E. R. Vedamuthu. 1969. Effects of medium and incubation temperature on recovery of microorganisms from manufacturing grade, Grade A, and pasteurized milk. J. Milk Food Technol. 32:90.

48. Heather, C. D., and C. Van Der Zant. 1957. Effect of temperature and time of incubating and pH of plating medium on enumerating heat-treated psychrophilic bacteria. J. Dairy Sci. 40:1072.

49. Heden, G. G., and R. W. G. Wychoff. 1949. The electron microscopy of heated bacteria. J. Bacteriol. 58:153.

50. Hedrick, T. I. 1970. How to keep milk fresh for three weeks. Am. Dairy Review. 32(4):56.

51. _____. 1971. Ways to achieve long shelf-life milk. Dairy Field 154:40.

52. Heineman, P. G. 1921. Milk. W. B. Saunders Co., Philadelphia and London. 684 p.

53. Heinmets, F., W. W. Taylor, and J. J. Lehman. 1954. The use of metabolites in the viability of heat and chemically inactivated Escherichia coli. J. Bacteriol. 67:5.

54. Huskey, J. E., J. E. Edmondson, and K. L. Smith. 1960. The effect of temperature on the keeping qualities of milk in market channels. J. Dairy Sci. 43:843. (abstr.).

55. Ingraham, J. L. 1958. Growth of psychrophilic bacteria. J. Bacteriol. 76:75.

56. Janzen, J. J. 1971. Flavor characteristics of normal and abnormal milk. J. Milk Food Technol. 34:352.

57. _____. 1972. The effect of somatic cell concentrations in the raw milk on the shelf-life of the processed product. J. Milk Food Technol. 35:112.

58. Jezeski, J. J. and H. Macy. 1946. Cryophilic organisms in water and butter. J. Dairy Sci. 29:439.

59. Johns, C. K. 1959. Applications and limitations of quality tests for milk and milk products. A Review. J. Dairy Sci. 42:1625.

60. _____. 1959. Detecting recontamination of pasteurized products. Can. Dairy and Ice Cream J. 38:34.

61. _____. 1960. Preliminary incubation for raw milk samples. J. Milk Food Technol. 23:137.

62. _____. 1962. Is a low milk count enough. J. Milk Food Technol. 25:375.

63. _____. 1968. Combatting milkstone on dairy farm equipment. J. Milk Food Technol. 31:21.

64. _____. 1969. Tests for estimating shelf-life of milk and milk products. J. Milk Food Technol. 32:301.

65. Kaufmann, O. W., and R. H. Andrews. 1954. The destruction rate of psychrophilic bacteria in skimmilk. J. Dairy Sci. 37:317.

66. Kaufmann, O. W., T. I. Hedrick, I. J. Pflug, C. G. Pheil, and R. A. Keppeler. 1960. Relative cleanability of various stainless steel finishes after soiling with inoculated milk solids. J. Dairy Sci. 43:28.

67. Kennedy, L., and H. Weiser. 1950. Some observations of bacteria isolated from milk that grow within the psychrophilic temperature range. J. Milk Food Technol. 13:353.

68. Lampert, L. M. 1970. Modern Dairy Products. Chemical Publishing Co., Inc., New York. 418 p.

69. Langlois, B. E., and A. W. Rudnick. 1969. Tests show need for more stringent quality controls. Am. Dairy Rev. 31:34.

70. Lawton, W. C., and F. E. Nelson. 1954. The effect of storage temperature on the growth of psychrophilic organisms in sterile and laboratory pasteurized skimmilk. J. Dairy Sci. 37:1164.

71. Leeder, J. G., and R. V. Martin. 1952. Effects of the hydrotron on milk and milkstone. J. Dairy Sci. 35:920.

72. Leggatt, A. G. 1970. The effect upon bacterial survival of sanitizer residues on a soiled surface. J. Milk Food Technol. 33:121.

73. Lewandowski, T. 1958. Laboratory study of factors influencing milkstone formation. J. Dairy Sci. 41:249.

74. Loane, P. C. 1969. A rapid test for determining the quality of raw refrigerated milks. Australian J. Dairy Technol. 6:66.

75. Luck, H. 1956. The use of hydrogen peroxide as a dairy preservative. Dairy Sci. Abstr. 18:363.

76. Macaulay, D. M., R. Z. Hawirko, and N. James. 1963. Effect of pasteurization on survival of certain psychrophilic bacteria. Applied Microbiol. 11:90.

77. Marth, E. H., and W. C. Frazier. 1957. Bacteriology of milk held at farm bulk cooling tank temperatures. I. Effects of time and temperature during storage, farm source of milk and season of year. J. Milk Food Technol. 20:72.

78. _____. 1957. Bacteriology of milk held at farm bulk cooling tank temperatures. II. Effect of numbers of bacteria in the original milk. J. Milk Food Technol. 20:79.

79. _____. 1957. Bacteriology of milk held at farm bulk cooling tank temperatures. III. Psychrophiles found and their growth. J. Milk Food Technol. 20:93.

80. Maxcy, R. B., and K. M. Shahani. 1961. Cleaning and sanitization of welded line systems for handling milk. J. Milk Food Technol. 24:122.

81. Maxcy, R. B. 1964. Potential microbial contaminants from dairy equipment with automated circulation cleaning. J. Milk Food Technol. 27:221.

82. _____. 1967. Nature and growth response of the microflora of pasteurized, packaged milk. J. Dairy Sci. 30:213.

83. _____. 1967. Rapid test for determining post-pasteurization contamination of market milk. J. Dairy Sci. 50:938.

84. _____. 1969. Determining post-pasteurization contamination by a surface plate technique. J. Milk Food Technol. 32:206.

85. _____. 1969. Residual microorganisms in cleaned-in-place systems for handling milk. J. Milk Food Technol. 32:140.

86. _____. 1971. Factors in the ecosystem of food processing equipment contributing to outgrowth of microorganisms on stainless steel surfaces. J. Milk Food Technol. 34:569.

87. McDonald, R. L. 1962. The utilization of several microbial tests to predict the quality and shelf-life of creamed cottage cheese. M. S. thesis. Texas Technological College, Lubbock.

88. McMeekin, T. L., and B. D. Polis. 1949. Milk Protein. Advances in Protein Chemistry. pp. 201-208. Academic Press Inc., New York.

89. Mikolajcik, E. M., and M. Koka. 1968. Bacilli in milk. I. Spore germination and growth. J. Dairy Sci. 51:1579.

90. Morrison, R. T., and R. N. Boyd. 1966. Organic Chemistry. p. 394. Allyn and Bacon, Inc., Boston.

91. Nageswararao, G., H. Blobel, and J. B. Derbyshire. 1969. Catalase activity for abnormal milk. I. Techniques and factors affecting the test. J. Milk Food Technol. 32:199.

92. Nageswararao, G., J. B. Derbyshire, and D. T. Berman. 1969. Sources and mechanism of catalase activity in the catalase test for abnormal milk. J. Milk Food Technol. 32:361.

93. Nashif, S. A., and F. E. Nelson. 1953. The lipase of Pseudomonas fragi. I. Characteristics of the enzyme. J. Dairy Sci. 36:459.

94. _____. 1953. The lipase of Pseudomonas fragi. II. Factors affecting lipase production. J. Dairy Sci. 36:471.

95. _____. 1953. The lipase of Pseudomonas fragi. III. Enzyme action in milk and cream. J. Dairy Sci. 36:481.

96. Nelson, F. E., and M. P. Baker. 1953. Influence of time and temperature of plate incubation upon bacterial counts of market milk and related products. J. Dairy Sci. 36:570.

97. _____. 1954. The influence of plate incubation upon bacterial counts of market milk and related products particularly after holding under refrigeration. J. Milk Food Technol. 17:95.

98. Nickolas, J. E., and T. G. Anderson. 1942. Keeping qualities of milk. Refrigeration Engineering. 43:370.

99. Nielsen, V. H. 1971. Controlling bacterial growth. Am. Dairy Rev. 33:32.

100. Olson, H. C. 1960. How to improve shelf-life. Proc. 57th Ann. Conv. Plant Section Milk Industry Foundation.

101. _____. 1960. The CVT test for detecting contamination in pasteurized milk. Correspondence, Oklahoma State University, Stillwater, Oklahoma.

102. _____. 1961. Selective media for detecting spoilage organisms in dairy products. J. Dairy Sci. 44:970.

103. _____. 1963. Selective plating technique for detecting contamination in pasteurized milk. J. Dairy Sci. 46:362.

104. _____. 1963. Spoilage of milk by thermoduric psychrophiles. J. Dairy Sci. 46:362.

105. Olson, H. C., G. O. Brown, and J. B. Mickle. 1960. A study of welded lines for processing milk. J. Milk Food Technol. 23:86.

106. Olson, H. C., and N. K. Chandhari. 1965. Comparison of methods for predicting the shelf-life of milk. J. Dairy Sci. 48:831.

107. Olson, H. C. 1967. Relationship between bacterial counts and shelf-life of milk. J. Dairy Sci. 50:607 (abstr.).

108. _____. 1971. Bacteriological testing of milk for regulatory purposes -- Usefulness of current procedures and recommendations for change. V. Pasteurized milk. J. Milk Food Technol. 34:279.

109. Olson, J. C., D. S. Willoughby, E. L. Thomas, and H. A. Morris. 1953. The keeping quality of pasteurized milk as influenced by the growth of psychrophilic bacteria and the addition of aureomycin. J. Milk Food Technol. 16:213.

110. Olson, J. C. 1962. Hygenic aspects of milk and payment for quality. J. Milk Food Technol. 25:355.

111. Orla-Jensen. 1921. Dairy Bacteriology. P. Blakeston's and Son Co., Philadelphia. 230 p.

112. Overcast, W. W., and J. D. Shean. 1959. Growth of certain lipolytic microorganisms at 4 C and their influence on free fatty acid and flavor of pasteurized milk. J. Dairy Sci. 42:1479.

113. Overcast, W. W., and G. A. Adams. 1966. Growth of certain psychrophilic bacteria in pasteurized milk as influenced by previous excessive psychrophilic growth in the raw milk. J. Milk Food Technol. 29:14.

114. Overcast, W. W. 1967. Extending shelf-life by controlling psychrophiles. Am. Dairy Rev. 29:42.

115. _____. 1968. Psychrophilic microorganisms and keeping quality of milk and its products. J. Dairy Sci. 51:1336.

116. Pack, M. Y., E. R. Vedamuthu, W. E. Sandine, and P. R. Elliker. 1968. Hydrogen peroxide-catalase treatment for enhancement and stabilization of diacetyl in lactic starter cultures. J. Dairy Sci. 51:511.

117. Palmer, L. S. 1926. Laboratory Experiments in Dairy Chemistry. John Wiley and Sons, Inc., New York. 84 p.

118. Patel, G. B. and G. Blankenagel. 1972. Bacterial counts of raw milk and flavor of the milk after pasteurization and storage. J. Milk Food Technol. 35:203.

119. Pennington, M. E., T. S. Hepburn, E. Q. St. John, E. Witner, M. O. Strafford, and J. I. Burrell. 1914. Bacterial and enzymic changes in milk and cream at 0 C. J. Biol. Chem. 16:331.

120. Pinheiro, A. J. R., B. J. Liska, and C. E. Parmelee. 1965. Heat stability of lipases of selected psychrophilic bacteria in milk and Purdue Swiss-type cheese. J. Dairy Sci. 48:983.

121. Prouty, C. C. 1955. Observations on the presence of facultative psychrophilic bacteria in milk produced under the farm tank system. J. Milk Food Technol. 18:250.

122. Punch, J. D., J. C. Olson, and E. L. Thomas. 1961. Preliminary observations on population levels of pure cultures of psychrophilic bacteria necessary to induce flavor and physical changes in pasteurized milk. J. Dairy Sci. 44:1160.

123. _____. 1965. Psychrophilic bacteria. III. Population levels associated with flavor or physical change in milk. J. Dairy Sci. 48:1179.

124. Randolph, H. E., T. R. Freeman, and R. W. Peterson. 1965. Keeping quality of market milk obtained at retail outlets and the processing plant. J. Milk Food Technol. 28:92.

125. Rigsby, P. D. 1955. A study of some of the factors affecting the quality and keeping quality of pasteurized-homogenized milk on the Lubbock, Texas retail market. M. S. thesis. Texas Technological College, Lubbock.

126. Rogick, F. A., and L. M. Burgwald. 1950. A study on psychrophilic bacteria in market milk. J. Dairy Sci. 33:403.

127. Sanhu, M. M. S. 1967. Contribution of the heat-resistant lipase of psychrophilic bacteria to off-flavor in pasteurized milk. M. S. thesis. University of Tennessee, Knoxville.

128. Schultze, W. D. 1959. An investigation of psychrophilic bacteria. Dissertation Abstr. 19:2434.

129. Schultze, W. D., and J. C. Olson. 1960. I. Distribution in stored commercial products. Studies on psychrophilic bacteria. J. Dairy Sci. 43:346.

130. Schumb, W. C., C. N. Gatterfield, and R. L. Wentworth. 1955. Hydrogen Peroxide. Reinbold Publ. Co., New York.

131. Shahani, K. M. 1966. Milk enzymes: Their role and significance. J. Dairy Sci. 49:90.

132. Sing, E. L. 1972. Program for quality assurance of finished dairy products. J. Milk Food Technol. 35:207.

133. Shean, J. D., and W. W. Overcast. 1960. Changes in the paper electrophoretic protein pattern of refrigerated skim milk accompanying growth of three Pseudomonas species. Appl. Microbiol. 8:335.

134. Slatter, W. L. 1971. Shelf-life of fluid milk. Paper distributed by John Lindawood, Extension specialist. Food Science and Nutrition Dept., Ohio State University.

135. Smille, D. M., M. J. Orr, and R. M. McLarty, 1958. Bulk milk collections in Wigtownshire. Bacterial Counts at 6 and 30 hours. Dairy Ind. 23:560.

136. Smith, A. C. 1968. The effect of filling temperature of milk on the keeping quality of the product. J. Milk Food Technol. 31:306.

137. Speck, M. L. 1961. The inactivation of bacteria in milk exposed to ultra-high pasteurization temperatures. J. Milk Food Technol. 24:358.

138. Stark, C. N., and B. J. Scheib. 1936. A study of fat-splitting and casein digesting bacteria isolated from butter. J. Dairy Sci. 19:191.

139. Storgards, T. 1961. Relationship between quality of producer's milk and market milk. Dairy Ind. 26:909.

140. Thomas, S. B., B. F. Thomas, D. G. Griffiths, and D. Ellison. 1953. Heat resistant bacteria in raw milk. VI. The seasonal incidence of thermoduric organisms. J. Dairy Sci. 36:A31 (abstr.).

141. Thomas, S. B., R. G. Druce, and Kay P. King. 1964. The microflora of steam sterilized milking equipment. J. Applied Bacteriol. 27:7.

142. Thomas, W. R., G. W. Reinbold, and F. E. Nelson. 1963. The effect of temperature and time of plate incubation on the enumeration of pasteurization-resistant bacteria in milk. J. Milk Food Technol. 26:357.

143. _____. 1966. Effect of the type of bacteriological peptone in the plating medium upon the enumeration of pasteurization-resistant bacteria in milk. J. Milk Food Technol. 29:182.

144. Thomas, W. R., G. W. Reinbold, and F. E. Nelson. 1966. Effect of pH of plating medium on enumeration of pasteurization-resistant bacteria in milk. J. Milk Food Technol. 29:156.

145. United States Department of Health, Education, and Welfare. Public Health Service. 1965. Grade "A" pasteurized milk ordinance. Washington, D. C. 185 p.

146. Van Der Zant, W. C., and A. V. Moore. 1955. The influence of certain factors on the bacterial counts and proteolytic activities of several psychrophilic organisms. J. Dairy Sci. 38:743 (abstr.).

147. Watrous, G. H., S. E. Barnard, and W. W. Collenan. 1971. A survey of the actual and potential bacterial keeping quality of pasteurized milk from 50 Pennsylvania dairy plants. J. Milk Food Technol. 34:145.

148. _____. 1971. Bacterial concentration in raw milk, immediately after laboratory pasteurization and following 10 days at 7.2 C. J. Milk Food Technol. 34:282.

149. Weber, F. J. 1958. Identification and physiological activities of psychrophilic microorganisms in milk. Dissertation Abstracts. 19:642.

150. Weese, S. J., and H. O. Henderson. 1949. The keeping quality of pasteurized milk in home refrigerators. J. Dairy Sci. 32:945.

151. Weese, S. J., D. F. Butcher, and R. O. Thomas. 1969. Effect of freezing and length of storage on milk properties. J. Dairy Sci. 52:1724.

152. Whitney, R. McL. 1958. The minor proteins in bovine milk. J. Dairy Sci. 43:1303.

153. Witter, L. D. 1961. Psychrophilic bacteria -- a review. J. Dairy Sci. 44:983.